VOLUME L NUMBER 2

BOTANICAL GAZETTE

AUGUST 1910

THE MORPHOLOGY OF THE PODOCARPINEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 138

MARY S. YOUNG

(WITH PLATES IV–VI)

Previous to 1902 the morphology of the Podocarpineae was an unknown field, but recently, through the contributions of COKER (**5**), JEFFREY and CHRYSLER (**7**), BURLINGAME (**2**), BROOKS and STILES (**1**), and YOUNG (**25**) on *Podocarpus* and *Dacrydium*, and NORÉN (**11**), STILES (**15**), THOMPSON (**16, 17, 18**), and TISON (**22**) on *Saxegothaea* and *Microcachrys*, the group as a whole has become fairly well known. *Pherosphaera*, with two species, is the the only genus as yet untouched, and therefore will not be considered in the following discussion.

One of the chief interests of the family is the question of relationships. The division of the Coniferales into the two families Taxaceae and Pinaceae was made originally on the basis of external characters, but with increasing knowledge of the Podocarpineae certain resemblances to the Araucarineae have been more and more emphasized, until it has even been suggested that these two tribes should form a group by themselves.

Three papers on *Phyllocladus* by Miss ROBERTSON (**13**) and Miss KILDAHL (**8, 9**) are of particular interest because of the difference of opinion in regard to the affinities of that genus. *Phyllocladus* was classed with the Podocarpineae by STRASBURGER, removed to the Taxineae by ENGLER and PRANTL, and finally made a sub-family by itself by PILGER (**12**) in 1906; and now, as its life history is studied, its place is again called in question. Miss ROBERTSON's work was

chiefly anatomical. Miss KILDAHL's work was done in this laboratory, and I have had the opportunity of examining her preparations. The receipt of additional material has made it possible to continue the study and to fill in some of the missing stages. This work, a previous study of *Dacrydium*, and the opportunity of examining some material of *Podocarpus* have given occasion for the present paper. I shall first consider *Phyllocladus*, and later take up the larger question of the relationships of the Podocarpineae.

I. Phyllocladus

The material sent by Dr. L. COCKAYNE of Christchurch, New Zealand, consisted of staminate cones collected at intervals of a few days from October 16 to November 13, and ovulate cones of November 8, 13, 25, December 3, 18, 31, January 8, 28. The iron-alum hematoxylin and orange combination was found very satisfactory in staining, as it brought out cytological details remarkably well for tissues killed in formalin and alcohol.

THE MALE GAMETOPHYTE

Miss KILDAHL reports (1) the formation of two prothallial cells, the first of which is usually evanescent; (2) the presence of four, occasionally five, free nuclei in the mature pollen grain; and (3) the division of the body cell into two equal male cells.

My material furnishes a close series, beginning with the microspore stage. The wings are comparatively small and irregular, recalling those of *Microcachrys*, and are formed in the ordinary way as cavities in the exine. The intine is usually rather thin. Very small starch grains occur, but are never conspicuous. Figs. 1–5 show the cutting off of the prothallial cells. The first usually degenerates so quickly that it is hard to find in the older grains. In fig. 4 it is seen partly covered by exine; this is an oblique section escaping the wings and making the spore coats appear uncommonly thick.

The generative cell is formed in the usual way and divides anticlinally, as in the case of *Ginkgo*, *Podocarpus*, and *Dacrydium*, and presumably in all the Podocarpineae. The four-celled stage, as shown in fig. 7, but for the wings might very easily be mistaken for the shedding stage in *Ginkgo*. In the latter, it will be remembered,

the generative cell does not divide until after the tube begins to grow. The spindle in *Phyllocladus* is always more or less oblique, and results in the formation of a larger, more centrally placed body cell and a small stalk cell.

In fig. 13 there is shown a peculiar but very common feature of the sections, the appearance as of an additional cell cut off from the body cell, but without a nucleus. This was noticed also in *Podocarpus* and *Dacrydium*. JEFFREY and CHRYSLER (**7**), in the case of *Podocarpus ferruginea* and *P. dacrydioides*, describe a second lateral derivative cell and picture it with a nucleus. BROOKS and STILES (**1**) find the same thing in *P. spinulosa*, but it is not clear whether a nucleus is present or not. It is in these species that the most extensive prothallial tissue is found, consisting of eight cells. In the forms studied by BURLINGAME and myself, as well as in *Dacrydium* and *Phyllocladus*, no nucleus ever appears in the extra section of cytoplasm. The explanation is most easily found in *Phyllocladus*, where horizontal sections show that it is really only a part of the stalk cell. When we consider the shape of the generative cell and the position of the spindle in the division, it is evident that an oblique wall in such a dome-shaped structure could not help resulting in the partial encircling of one cell by the other. The situation can be most clearly shown by diagrams. A horizontal section in the position shown by the dotted line *xy* in fig. 15 would give a view such as is outlined in fig. 16, while fig. 15 is the vertical section through the dotted line in fig. 16. Fig. 17 is an oblique section in about the position of the dotted line *vw* in fig. 15. In this case the second prothallial cell is seen encircled by the stalk cell. A change of focus brings into view the body and tube nuclei and a portion of one wing.

If the stalk nucleus, as shown in fig. 16, were a little more elongated, it would be quite possible to obtain a vertical section in such a position as to show a small part of it on each side of the body cell. The appearance of JEFFREY and CHRYSLER'S and of BROOKS and STILES'S figures of *Podocarpus ferruginea*, *P. dacrydioides*, and *P. spinulosa* suggests this as a possible explanation of the second lateral derivative.

The fact has been noted that the division of the generative cell is anticlinal in Podocarpineae and *Phyllocladus*, and periclinal in other conifers and in cycads, the relative position in the latter having given

rise to the term "stalk cell." It will be remembered that the division takes place in the former before and in the latter after germination has begun. Fig. 18 is a diagram showing this stage in *Dioon*, taken from CHAMBERLAIN (**3**). If in the case of *Phyllocladus* the generative cell should enlarge upward toward the tube before the division, we can easily see that the oblique wall, failing to touch the prothallial cell, would give us the *Dioon* situation. On the other hand, if the wall in *Dioon* were a little lower down or slightly more oblique, we should have a section very like that of *Phyllocladus*. From this point of view the distinction between the anticlinal and periclinal division appears to be related to the time at which the division takes place, and to be of little significance in itself.

Throughout the development of the gametophyte, though distinct cells are formed, each bounded by a *Hautschicht*, there is no evidence of cellulose walls. This I believe to be true also in the *Dacrydium* and *Podocarpus* which I have examined. NORÉN (**11**) fails to find cellulose walls in the case of *Saxegothaea* except in the first prothallial cell, and THOMPSON (**17**) finds them in *Microcachrys* only in the prothallial cells.

Miss KILDAHL mentions the occasional persistence of the first prothallial cell. Figs. 11 and 12 show not only this but also a still more rare case in which the second has divided. This, it will be remembered, is the usual condition in *Dacrydium*, *Microcachrys*, and *Saxegothaea*. In *Dacrydium*, moreover, the first prothallial cell very often degenerates early, which makes the resemblance to *Phyllocladus* still stronger.

As in all the Podocarpineae so far studied, the prothallial and stalk nuclei become free in the general cytoplasm. This may occur in *Phyllocladus* before the grains are shed, but sometimes not until after they reach the micropyle. The mature grain contains the body cell, and the free prothallial, stalk, and tube nuclei.

Young tubes were found in the nucellus November 20. Fig. 19 shows the tube nucleus in advance, followed by the others, and the distinctly organized body cell still in the grain.

My work does not confirm Miss KILDAHL'S in regard to the male cells, as in every case where a complete series of sections was secured, a decided difference in size was evident. The functional

cell is almost always in advance. It has not only more cytoplasm than the other, but nearly always a larger nucleus, and often the other shows signs of degeneration. Figs. 20 and 32 are both drawn so as to show the largest diameter of each male cell and nucleus. The division of the cytoplasm is sometimes hard to demonstrate, and the appearance is that of two free nuclei. Careful staining, however, shows that two definite male cells are formed.

THE FEMALE GAMETOPHYTE

Some belated cones collected November 18 gave the earliest stages. Fig. 21 shows an ovule with its free nucellus, its wide open micropyle with pollen grains, and the arillus making its appearance as a slight swelling at the base. The stony part of the integument develops from a layer two cells deep. The "outer fleshy layer" does not thicken up, but remains represented by only the epidermis and the cells directly under it.

The megaspore mother cell evidently gives rise to a row of three cells, the innermost being the functional megaspore; in fig. 3 it has germinated and has two free nuclei. The young prothallus is surrounded by a layer of glandular, vacuolate cells encroaching on the surrounding nucellar tissue. This "spongy layer" at a later period is much more marked and the cells become binucleate. The megaspore membrane soon appears and is well marked in the early free-nucleate stages. In having the megaspore membrane and spongy layer *Phyllocladus* agrees with all the Podocarpineae except *Podocarpus*, and differs from all the Taxineae.

Wall formation was not found, nor archegonium initials. The development of the young gametophyte is evidently rapid, for material collected November 25 was in the free-nucleate stage, while that of December 3 contained archegonia and an occasional very young embryo.

The gametophyte produces two archegonia, each with its jacket of multinucleate cells; Miss KILDAHL found occasionally three or four. The youngest archegonium obtained is shown in fig. 24. The width at the top seems to be due to the rapid centrifugal growth of the tissues at the upper (archegonial) end of the prothallus, which is indicated by radial lines of cells and numerous spindles. The initial

has divided, forming a central cell and a primary neck cell; the latter gives rise by two divisions to a plate of four cells (figs. 25–27). An exceptional case is seen in fig. 29, where a division has occurred in each of the four cells. Cellulose walls (in this division), however, are not formed, the cells being separated merely by a *Hautschicht*, and in one of them not even a *Hautschicht* appears. The cytoplasm of the central cell is very delicate in the early stages, but gradually thickens up. The nucleus is rather small and lies at the upper end close under the neck.

There is an interesting peculiarity in the neck development of *Phyllocladus* which, so far as I know, has not been reported in any other genus. Miss KILDAHL described the pollen tube as pushing its way into the archegonium with two apparently detached cells in front of it, which she thought were the remains of a crushed neck. Fig. 32 shows this stage, but there are four neck cells, and they are connected with the adjacent jacket cells by a distinct membrane. This membrane at first sight is easily mistaken for the wall of the pollen tube, but the latter is very thin and usually shrinks away from the archegonium in the preparations.

The origin of this peculiar condition is found in a series of earlier stages. The young archegonia are superficial and the necks are covered by the heavy megaspore membrane (figs. 24–26). The pollen tubes at this time come in contact with the prothallus near the necks, and the adjacent tissues grow rapidly, leaving the archegonia at the bottom of considerable cavities which are occupied by the tubes (fig. 36). In *Cephalotaxus*, according to COKER (**4**), the archegonia, in the absence of pollination, may be entirely inclosed by the growth of the lateral tissue. The megaspore membrane is always a little thinner at the micropylar end, but disappears entirely inside the archegonial cavities (fig. 37), apparently digested by the male gametophyte. Fig. 27 shows it beginning to disappear under the advancing tube. The growth of the female tissues and the pressure of the tube result in a lateral stretching, first of the neck, then of the adjoining jacket cells (fig. 28). The walls of these latter are able to resist the digestive action and enlarge enormously, until just before fertilization they appear as the membrane mentioned above (figs. 33–35).

An interesting situation is shown in fig. 34. The ovule contained

three archegonia, of which the two shown had apparently arisen from adjacent initials and formed a complex with a common jacket. The necks, consisting of four cells each, are attached to one another, one on each side of the common wall. This wall is almost parallel with the plane of the page and passes between the two egg nuclei. In another gametophyte three archegonia were found with a single jacket layer separating two of them; the usual amount of tissue lay between them and the third.

Fig. 33 illustrates the resistance of the jacket membrane to the action of the tube. The neck of this archegonium has been pushed into a vertical position, and part of the egg cytoplasm has been squeezed away from the rest and shows signs of degeneration.

Shortly before fertilization a ventral canal nucleus is cut off, but there is no trace of a wall (figs. 30, 34). In this *Phyllocladus* agrees with *Podocarpus*, *Taxus*, and *Cephalotaxus*, but not with *Torreya*. In the latter not even a nucleus has been found.

Podocarpus is the only member of its tribe whose female gametophyte has been studied. In it COKER (**5**) found six to ten archegonia. The neck varies greatly, having commonly three tiers of four cells each, but sometimes the number of cells is more than twenty-five.

FERTILIZATION AND THE EMBRYO

At the time of fertilization both egg and jacket cells are rich in "proteid vacuoles," and the egg nucleus is surrounded by a homogenous and very dense layer of cytoplasm. The contents of the pollen tube enter the egg through the neck, leaving the cells intact. Fig. 35 shows both male cells in the egg; the larger fuses with the egg nucleus, some of the cytoplasm apparently contributing to the embryo. Though no trace of the prothallial, stalk, and tube nuclei could be found in this case, it is reasonable to suppose that they entered with the rest of the contents of the tube. In fig. 27 a tube is seen pressing against the side of an archegonium. With further growth probably the wall will be forced into a vertical position, in which case fertilization can take place in the usual way.

Miss KILDAHL reports the formation of at least eight free nuclei in the proembryo, and my material furnishes nothing more. Ovules collected in January had embryos with two cotyledons and long

suspensors. The entire endosperm tissue is multinucleate, a condition which begins to appear while the archegonia are young; it results from the failure of walls to form. In the older endosperm as many as eight nuclei occur in a cell. The megaspore membrane at the fertilization period is about 2.5 μ thick at the lower part of the prothallus, and about 4.5 μ when the cotyledons appear (fig. 31).

Before discussing relationships, it will be helpful to summarize the important facts about *Phyllocladus*, including the work of Miss ROBERTSON and Miss KILDAHL.

SUMMARY

1. The stamen bears two abaxial sporangia.
2. The pollen grains have two wings.
3. In the male gametophyte there are two prothallial cells; the first usually disappears, the second occasionally divides.
4. The generative cell divides anticlinally but obliquely, and the stalk cell partially encircles the second prothallial and body cells.
5. The mature pollen grain contains the body cell and the free stalk, prothallial, and tube nuclei.
6. There are two unequal male cells, only the larger of which functions.
7. The ovulate structure is a strobilus with a single erect ovule in the axil of each scale.
8. A symmetrical arillus originates from the base of the ovule and remains free from the integument.
9. The outer fleshy layer is represented by two layers of cells.
10. The nucellus is free to the base.
11. The megaspore mother cell gives rise to a row of three cells, the innermost of which is the functioning megaspore.
12. The spongy layer and megaspore membrane are strongly developed.
13. Each ovule contains two archegonia, occasionally three or four, each with its own jacket, but sometimes there is a complex of two in a common jacket.
14. The neck of the archegonium consists of four cells in a plate; occasionally these divide anticlinally.
15. There is a ventral canal nucleus.

16. The mature archegonium is very wide at the top and is covered by a membrane formed from the walls of the adjacent jacket cells.

17. In fertilization the contents of the pollen tube pass through the neck, leaving it intact.

18. Some male cytoplasm contributes to the embryo.

19. There are at least eight free nuclei in the proembryo.

20. The embryo has two cotyledons and a very long suspensor.

21. Mesarch bundles occur in the cladodes.

22. Taxinean sculpturing is found in the tracheids.

23. No vascular strands enter the ovule; the ovular supply consists of two strands facing each other and ending in a tracheal plate below the integument.

DISCUSSION

The characters which point toward the affinity of *Phyllocladus* with the Taxineae are: (1) the structure of the ovule, (2) the symmetrical arillus, (3) resemblances of the ovulate cone to that of *Cephalotaxus*, (4) mesarch bundles, and (5) taxinean sculpturing of tracheids. Characters indicating a relationship with the Podocarpineae are: (1) the character of the male gametophyte, (2) the structure of the stamen, (3) winged pollen grains, (4) megaspore membrane and spongy tissue.

The first point is perhaps the strongest argument for taxad affinity, as the erect, free, axillary ovule is characteristic of the whole group. This ovule, however, is a primitive type which we would expect to find at the base of any line. Though the progress of the podocarps has been toward inversion and fusion of parts, which finds its highest expression in *Podocarpus* itself, we find many relatively primitive stages represented. In *Dacrydium*, *Saxegothaea*, and *Microcachrys* the ovule is free from the scale and epimatium, except at the base, and in the various species of *Dacrydium* we find all positions from erect to completely inverted. The young ovule of *Saxegothaea* is in early stages perpendicular to the scale and becomes inverted only as the result of later growth. This, together with its position near the base of the scale, is suggestive of ancestors with erect, axillary ovules.

The homology of the symmetrical arillus which originates from

the base of the ovule, and of the one-sided epimatium arising from the scale is uncertain. The origin of the latter may be related, however, to the foliar origin of the ovules, and, as Miss ROBERTSON states, "the asymmetry is correlated with the inverted position of the ovule, so that it will not do to lay too much stress on this point, as proving that epimatium and arillus are not homologous."

Though the external resemblance between the cones of *Phyllocladus* and *Cephalotaxus* is rather striking and the vascular supply is the same, this may merely point back to a common origin of all the Taxineae. *Cephalotaxus*, moreover, has two ovules in the axil of each bract, while *Phyllocladus* agrees with the Podocarpineae in having only one.

The presence of mesarch wood in the cladodes owes its significance to the fact that mesarch wood is more common in Taxineae than in any other group of conifers. This, too, is an ancestral character, for which we would look in the primitive members of any group. STILES finds it in the ovular supply in *Saxegothaea*, whose cone is regarded as the most primitive of the podocarps.

Taxinean sculpturing of tracheids, too, would be more significant if it were confined to Taxineae, but this is by no means true. Such characters as these last two are valuable in connection with other evidence, but have little weight in themselves.

The most convincing evidence of podocarp affinity lies in the entire behavior of the male gametophyte: the formation of prothallial tissue, the freeing of the nuclei, and the early division of the generative cell. The presence or absence of prothallial cells is a definite group character; where they occur at all they are characteristic of an entire family, and where absent in one member they are lacking in all. Permanent prothallial cells are known nowhere in conifers except in Podocarpineae and Araucarineae; and the early division of the generative cell, which characterizes the former, occurs nowhere else except perhaps in araucarians. Prothallial tissue is a primitive character, possessed, as we presume, by the ancestors of all conifers; but the Taxineae have eliminated it entirely, while it still remains one of the most characteristic features of the Podocarpineae.

The morphology of the different forms of stamen in the conifers is an open question. The Taxineae, according to COULTER and

LAND (**6**), are reducing the epaulette type of *Taxus*, while no other family, except araucarians, has a stamen with more than two pollen sacs. If the two types have a common origin, only the *Taxus* stamen can be the primitive one; and in this case *Phyllocladus* has gone a long way in the podocarp line of development. A different origin, of course, would remove *Phyllocladus* still farther from Taxineae.

Winged pollen is entirely absent among the Taxineae, but is characteristic of the Podocarpineae, being absent only in the *Saxegothaea*. The irregularity in size and number of the wings in *Microcachrys* has given rise to THOMPSON'S (**16, 17**) theory that the two-winged condition has developed within the group and shows no relation to Abietineae. This gives us another podocarp line along which *Phyllocladus* has advanced.

THOMPSON (**20**) lays a good deal of stress on the megaspore membrane as a primitive character. It is entirely eliminated in Taxineae, but characterizes all the members of the other family except *Podocarpus* itself. As the ovulate structures of the type genus are by far the most specialized of the family, this is not surprising. Miss ROBERTSON thought the megaspore membrane and spongy tissue were in some way correlated with the presence of winged pollen, but their occurrence in *Saxegothaea* with its wingless microspores breaks down this supposition.

CONCLUSION

When we consider the question of relationships, it is evident on the whole that: (1) *Phyllocladus* has primitive characters of the Taxineae which are being eliminated in the Podocarpineae; (2) it has primitive characters of the Podocarpineae which have been entirely eliminated in the Taxineae; (3) it has some advanced characters of Podocarpineae; (4) the taxad resemblances are on the whole more superficial and variable, and the podocarp features more fundamental; (5) the resemblances to Podocarpineae are too strong to justify the retention of the intermediate family.

We conclude, therefore, that *Phyllocladus* is a relatively primitive member of the Podocarpineae, which branched off from them a comparatively short time after their separation from Taxineae. Whether the two families form a distinct line from other conifers is not at all involved in this conclusion.

II. Podocarpineae and Araucarineae

The more important contrasts between the araucarian and podocarp lines are in the structure of the stamen and ovulate strobilus, and in the method of fertilization. The araucarian stamen is a comparatively primitive type and is somewhat suggestive of *Ginkgo*. It is one-sided and bears three to thirty pendent pollen sacs; while all the genera of the Podocarpineae, as before mentioned, have the microsporangia definitely reduced to two.

The ovulate cone in Araucarineae is a compact structure with many scales, ripening dry; while the podocarp line is characterized by the reduction in the number and size of cone scales and the tendency to fleshy development. *Saxegothaea*, the form which stands nearest the araucarians, shows the least amount of reduction in both size and number of scales; *Microcachrys* is next; while in other forms the cone is represented by the single, apparently terminal, ovule and a few rudimentary scales. The podocarp ovules have the arillus, or epimatium, which is absent in araucarians. The cone scale of *Araucaria*, on the other hand, bears the so-called ligule, represented in *Agathis* by only a slight projection from the surface. The ovules of *Saxegothaea* are united with the scale only at the base, while in *Araucaria* they are described as imbedded in the tissues of the scale. In *Agathis*, however, the ovules are free and the seeds winged.

The female gametophyte is too little known in either group for any adequate comparison. That of the Araucarineae, however, is apparently much more primitive than that of the known podocarps. The archegonia are very numerous and are described (SEWARD and FORD **14**) as situated at the bottom of deep pits and usually not connected with the surface by necks. Investigation is needed here to show whether they are really hypodermal, or whether the condition is brought about by overgrowth of adjacent tissue in the development of a neck so massive as not to have been recognized. The position of the ovule of Araucarineae in the tissues of the scale is another subject which needs interpretation.

Fertilization in Araucarineae (THOMPSON **19**) is angiosperm-like, in that the pollen does not reach the ovule, the tubes growing over the surface of the scales to reach the micropyle.

The first suggestion of relationship between the two groups came from the study of the male gametophyte. This has recently been supplemented by studies of *Saxegothaea,* which have brought out striking resemblances to certain species of araucarians. The geographic distribution is also suggestive of alliance between these two great southern groups.

THE MALE GAMETOPHYTE

In the Araucarineae, as in the Podocarpineae, there are two original prothallial cells, from which by subsequent divisions a more or less extensive tissue is formed. In Araucarineae there may be as many as thirty cells (LOPRIORE **10**; THOMPSON **21**). JEFFREY and CHRYSLER (**7**) found an apparently mature pollen grain of *Agathis australis* with a prothallial complex of eight cells, which is the situation in some species of *Podocarpus.* Other species of *Podocarpus* have four cells, resulting from a single division of each original prothallial cell. In *Dacrydium, Saxegothaea,* and *Microcachrys* there are two to four permanent prothallial cells, in *Phyllocladus* one to three. Thus we have a complete overlapping prothallial reduction series from *Agathis* to *Phyllocladus.*

The generative cell is found in Araucarineae as in the others, but the division into stalk and body cell has not been observed. Unless this division takes place in the tube, as is true of most conifers, it must either have been eliminated or missed in the preparations. The otherwise close correspondence with the gametophyte of Podocarpineae inclines one toward the latter supposition. Further work is needed on this point.

THE OVULE AND FEMALE GAMETOPHYTE

The single inverted ovule is characteristic of both families. The inversion was probably developed independently, however, in the two lines. In *Saxegothaea,* as has been said before, the young ovule changes from an erect to an inverted position in the course of its development. The free nucellus of *Dacrydium* and *Phyllocladus* is a primitive feature which they have in common with araucarians.

The female gametophyte, as before stated, is little known. A

point of resemblance, however, between Araucarineae, *Phyllocladus*, and *Podocarpus* is found in the cutting off of the ventral canal nucleus in the archegonium.

SAXEGOTHAEA

The resemblance of *Saxegothaea* to the araucarians, noted by STILES (**15**), NORÉN (**11**), THOMPSON (**18**), and TISON (**22**), consists chiefly of anatomical characters and certain external features of the cones. STILES finds in the stem tracheids somewhat araucarian in character, "an occasional tendency to a two-ranked arrangement of the pits, and in these cases the pits become alternate and hexagonal." Two-ranked and alternate pits horizontally flattened have also been found in *Dacrydium*. The prominence of transfusion tissue is another character suggestive of araucarian affinity.

The staminate cone is described by STILES as somewhat araucarian in general appearance, and the wall of the microsporangium as strikingly like that of *Araucaria Rulei*. BROOKS and STILES (**1**), in their study of *Podocarpus spinulosa*, state that the wall of the sporangium is very like that of *Saxegothaea* and *Araucaria*. Wingless pollen is another point of contact. The fact that the pollen grains of all the other Podocarpineae have wings does not affect the argument, if we accept THOMPSON'S theory that they were developed within the group (**15, 16**).

In the ovulate cones of both *Saxegothaea* and *Araucaria*, especially *A. Rulei*, there is a gradual transition from foliage leaves to sporophylls. This and the similarity in the vascular anatomy are considered by THOMPSON and TISON as indications of the simple nature of the strobilus in these groups. The occurrence of a single resin duct in the sporophyll is, according to STILES, another indication of this in *Saxegothaea*.

One of the most striking features of *Saxegothaea* is the projection of the nucellar tissue through the micropyle, where it expands to form a stigma-like knob. The same thing occurs less conspicuously in Araucarineae, but, with the exception of a few abnormal cases in angiosperms, is elsewhere unknown. This feature appears to be related physiologically to the difficulty of fertilization, and its morphological significance is doubtful. THOMPSON (**19**) sees in it a

tendency toward protosiphonogamic fertilization, significant in relating *Saxegothaea* to the araucarians.

A good deal of stress has been laid recently on the distribution of the vascular bundles in the ovulate sporophylls. STILES finds the branching in *Saxegothaea* very similar in the main to *Araucaria Rulei* and *A. Cookei*, and the arrangement in *Microcachrys*, though not unlike that of *Saxegothaea*, resembles more closely *A. Bidwillii*. TISON agrees essentially with STILES in regard to the details in *Saxegothaea*, but finds greater resemblance to *A. brasiliana* and *A. imbricata*.

RESULTS

STILES, NORÉN, THOMPSON, and TISON all agree in the relationship of the two groups, the intermediate position of *Saxegothaea*, and the simple structure of the ovulate scale in the two families, but they differ somewhat in the application of their results. STILES says: "While we must wait for fuller knowledge before making any definite statement, it would appear that these two orders of Coniferales are descended from some common ancestor with its micro- and megasporophylls both arranged spirally in cones. Along one line of descent we find the Araucarieae, along the other is *Saxegothaea* leading on to *Microcachrys* and the other Podocarpeae." NORÉN comes to the same conclusion, regarding *Saxegothaea* as nearest to the Araucarineae, but unquestionably a member of the Podocarpineae; but TISON considers it more closely related to the latter group, and regards *Microcachrys* as the connecting link.

But it is the attempt to homologize the ovulate structures of conifers that has given rise to the greatest amount of discussion. In conifers with foliar ovules, as is well known, there is more or less completely represented a double system of bundles, that from which the ovule gets its supply being inverse to the other. The two systems may originate separately from the axis, or the one supplying the ovule may arise at various situations from the other. The former varies also in extent, from a considerably branched system to one or two weakly differentiated bundles.

WORSDELL (**23**) in 1899 made a study of the distribution of the vascular bundles in various conifers, and in 1900 (**24**) published a

very complete historical account of the controversy on the morphology of the ovulate cone. He agrees with ČELAKOVSKÝ that the arillus of Taxaceae, the ligule of *Araucaria*, and the ovuliferous scale of the other Pinaceae are homologous and are all a second integument, that all the ovulate cones are morphologically compound, but that the sporophyll is suppressed and represented by the ovule alone.

THOMPSON (**18**) in 1909 brought out some interesting data in regard to the inversion and its relation to the theory of the axillary shoot. The inversion of the bundles supplying the ovule he explains as normal for sporangial supply, and cites cases of such inversion in the microsporophylls of cycads and of some conifers. In *Tsuga* he found two inversions, the ovular supply being inverse to that of the scale, which in turn is inverse to that of the bract. The first inversion he considers as homologous with that of the scale of *Saxegothaea* and related to the ovule, while the second may be explained by the theory of the compound nature of the sporophyll. On this basis he makes two groups of conifers, the Araucarineae and Podocarpineae having simple strobili and ovules on the morphological upper surface of the scale, and the other Pinaceae having compound strobili and ovules morphologically abaxial.

TISON regards the arillus of *Saxegothaea* and the ligule of *Araucaria* as homologous with the ovuliferous scale of other conifers, calling them an ovuliferous appendage. He does not commit himself in regard to the axillary shoot theory as affecting Abietineae, Cupressineae, and Taxodineae, but agrees with THOMPSON that the cones of *Saxegothaea*, Podocarpineae, and Araucarineae are simple. He favors the inclusion of *Saxegothaea* and Podocarpineae in the Araucariales suggested by SEWARD and FORD (**14**).

CONCLUSION

After reviewing the whole situation, one is impressed with the fact that both Podocarpineae and Araucarineae are very primitive, that they are probably related, but that the question is by no means settled. The whole coniter group still appears as a maze of cross resemblances. If we confine ourselves to one or two characters, the problems of relationship are comparatively simple, but bring more into consideration, and we are immediately in trouble. The

relation of the podocarps with Araucarineae on the one hand, for instance, is complicated with evidence of connection through *Phyllocladus* with taxads on the other; and the two last-named tribes are apparently very widely separated from one another. It is true that the evidence for the first relation is the stronger, for without *Phyllocladus* the question of taxad affinity would hardly be raised at all; while without *Saxegothaea* we should still have much evidence of the other connection. The existence of *Phyllocladus*, however, cannot be disregraded.

On the other hand, the argument for araucarian connection lacks conclusiveness. In the first place, it is based largely on primitive characters. These may indicate merely that neither group has advanced far from the original ancestral conifer stock. The two lines may be quite distinct and both short. Two short branches from the same trunk may have their ends nearer together than two twigs of the same long branch. Most of the remaining evidence depends upon the external features of the cones and the distribution of vascular bundles. Such evidence is unsatisfactory on account of the great variability of the structures concerned. Great variations are found between closely related forms. *Araucaria* with its ligule and apparently imbedded ovule, and *Agathis* with its free winged seed, or the ovulate structures of different species of *Dacrydium*, are examples. TISON finds variations in the arrangement of the vascular bundles of *Saxegothaea*, not only in different individuals, but in the same plant. He says: "En ce qui concerne les écailles fertiles, je dois tout d'abord faire remarquer que la disposition du système fasciculaire à leur base, dans la région ovulifère, est très variable souvent dans un même cône, ces variantes n'étant pas nécessairement en rapport avec la position des bractées sur les cônes."

The fact of variability makes it easy to imagine the derivation of a structure from one unlike it in appearance, as the arillus and ligule or ovuliferous scale; but on the other hand, their independent origin becomes equally simple, and one is inclined to doubt the morphological need of such homologies. It may be that the conflicting lines of resemblance among conifers point to a more ancient lineage for all the families than we are apt to think, and in this case the significance of variable features becomes still less.

The greatest gap in our present knowledge of conifers is shown by the Araucarineae. Even in the male gametophyte, which supplies perhaps the strongest argument in the above discussion, a good deal of work is needed. The female gametophyte is little known and the embryo practically not at all. Beside this, we should know the early development of the ovulate strobilus for a proper understanding of the morphology of its parts. In the Podocarpineae we lack adequate knowledge of the female gametophyte, embryo, and development of ovulate structures. Until further data on these points are available, we should be hardly justified in coming to a definite decision in regard to relationships, and at present it seems best to hold Taxineae, Podocarpineae, and Araucarineae apart as separate tribes, leaving open the question of larger groupings among conifers.

Grateful acknowledgments are due to Professors J. M. COULTER and C. J. CHAMBERLAIN, under whose direction this work was done.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BROOKS, F. T., and STILES, W., The structure of *Podocarpus spinulosa* (Smith) R. Br. Annals of Botany **24**:305–318. *pl. 21.* 1910.
2. BURLINGAME, L. L., The staminate cone and male gametophyte of *Podocarpus*. BOT. GAZETTE **46**:161–178. *pls. 8, 9.* 1908.
3. CHAMBERLAIN, C. J., Spermatogenesis in *Dioon edule*. BOT. GAZETTE **47**:215–236. *pl. 15.* 1909.
4. COKER, W. C., Fertilization and embryogeny in *Cephalotaxus Fortunei*. BOT. GAZETTE **43**:1–10. *pl. 1.* 1907.
5. ———, Notes on the gametophytes and embryo of *Podocarpus*. BOT. GAZETTE **33**:89–107. *pls. 5–7.* 1902.
6. COULTER, J. M., and LAND, W. J. G., Gametophytes and embryo of *Torreya taxifolia*. BOT. GAZETTE **39**:161–178. *pls. A, 1–3.* 1905.
7. JEFFREY, E. C., and CHRYSLER, M. A., The microgametophyte of the Podocarpineae. Amer. Nat. **41**:355–364. 1907.
8. KILDAHL, N. J., The morphology of *Phyllocladus alpina*. BOT. GAZETTE **46**:339–347. *pls. 20–22.* 1908.
9. ———, Affinities of *Phyllocladus*. BOT. GAZETTE **46**:464–465. 1908.
10. LOPRIORE, G., Ueber die Vielkernigkeit der Pollenkörner und Pollenschläuche von *Araucaria Bidwillii*. Ber. Deutsch. Bot. Gesell. **23**:335–346. *pl. 15.* 1905.

11. NORÉN, C. O., Zur Kenntniss der Entwickelung von *Saxegothaea conspicua* Lindl. Svensk. Botanisk. Tidskrift **2**:101–122. *pls. 7–9.* 1908.
12. PILGER, R., Taxaceae in Das Pflanzenreich (ENGLER) **4**:5. 1903.
13. ROBERTSON, A., Some points in the morphology of *Phyllocladus alpina* Hook. Annals of Botany **20**:259–265. *pls. 17, 18.* 1906.
14. SEWARD, A. C., and FORD, S. O., The Araucarieae, recent and extinct. Phil. Trans. Roy. Soc. London B **198**:305–411. *pls. 23, 24.* 1906.
15. STILES, W., The anatomy of *Saxegothaea conspicua* Lindl. New Phytologist **7**:209–222. 1908.
16. THOMPSON, R. B., Note on the pollen of *Microcachrys*. BOT. GAZETTE **46**:465. 1908.
17. ———, On the pollen of *Microcachrys tetragona*. BOT. GAZETTE **47**:26–29. *pls. 1, 2.* 1909.
18. ———, The megasporophylls of *Saxegothaea* and *Microcachrys*. BOT. GAZETTE **47**:345–354. *pls. 22–25.* 1909.
19. ———, The Araucarieae; a protosiphonogamic method of fertilization. Science N.S. **25**:271, 272. 1907.
20. ———, The megaspore membrane of the gymnosperms. Univ. Toronto Studies, Biol. Ser. no. 4. *pls. 1–5.* 1905.
21. ———, Preliminary note on the Araucarineae. Science N.S. **22**:85. 1905.
22. TISON, A., Sur le *Saxegothaea conspicua* Lindl. Mém. Soc. Linn. Normandie **23**:139–160. *pls. 9, 10.* 1909.
23. WORSDELL, W. C., Observations on the vascular anatomy of the female "flowers" of Coniferae. Annals of Botany **13**:527–548. *pl. 27.* 1899.
24. ———, The structure of the female "flowers" in Coniferae. Annals of Botany **14**:39–82. 1900.
25. YOUNG, M. S., The male gametophyte of *Dacrydium*. BOT. GAZETTE **44**:189–196. *pl. 19.* 1907.

EXPLANATION OF PLATES IV–VI

Zeiss compensating oculars 4 and 6 mm. and Bausch and Lomb objectives $\frac{1}{8}$, $\frac{1}{12}$, and $\frac{1}{16}$ mm. were used. All drawings, except figs. 15, 16, and 18, were made with the aid of the camera lucida. The magnifications refer to the figures as they appear on the plates, after having been reduced one-half in reproduction.

Abbreviations: p_1, first prothallial cell; p_2, second prothallial cell; *g*, generative cell; *s*, stalk cell; *b*, body cell; *t*, tube nucleus; *a*, arillus; *n*, neck; m_1, functioning male cell; m_2, functionless male cell; *vn*, ventral canal nucleus; *e*, egg nucleus; *m*, megaspore membrane; *sp*, spongy tissue.

FIG. 1.—Microspore. ×950.

FIG. 2.—Spindle for the first prothallial cell. ×950.

FIG. 3.—First prothallial cell cut off. ×950.

FIG. 4.—Spindle for the second prothallial cell; first prothallial cell partly covered by intine. ×950.

FIG. 5.—Second prothallial cell cut off, the first degenerating. ×950.

FIG. 6.—Spindle for generative cell. ×950.

FIG. 7.—Tube nucleus, generative cell, and prothallial cells. ×950.

FIGS. 8, 9.—Generative cell dividing. ×950.

FIG. 10.—Mature pollen grain. ×950.

FIGS. 11, 12.—First prothallial cell persistent and the second divided. ×950.

FIG. 13.—Mature grain, showing a part of the stalk cell on each side of the body cell. ×950.

FIG. 14.—Nuclei becoming free. ×950.

FIG. 15.—Diagram of vertical section through dotted line in fig. 16.

FIG. 16.—Diagram of horizontal section through dotted line *xy* of fig. 15, showing form of stalk cell.

FIG. 17.—Horizontal section, showing stalk and second prothallial cells and tube and body nuclei. ×950.

FIG. 18.—Outline drawing of *Dioon*.—From CHAMBERLAIN.

FIG. 19.—Body cell in the grain; stalk, tube, and prothallial nuclei in the tube. ×440.

FIG. 20.—Body cell divided, forming two unequal male cells. ×440.

FIG. 21.—Longitudinal section of ovule and bract; pollen grains on the nucellus. ×45.

FIG. 22.—Young stage of the arillus; detail of fig. 21. ×440.

FIG. 23.—Two-nucleate stage of female gametophyte; detail of fig. 21. ×440.

FIG. 24.—Young archegonium, showing primary neck and central cells. ×440.

FIG. 25.—Young archegonium; primary neck cell divided. ×440.

FIG. 26.—A neck cell dividing. ×440.

FIG. 27.—Four-celled neck; megaspore membrane disappearing before advancing pollen tube; the space between the membrane and neck cells is due to shrinkage. ×440.

FIG. 28.—Stretching of adjacent jacket cells under pressure of the pollen tube; body cell and central nucleus not yet divided. ×440.

FIG. 29.—Eight-celled neck. ×440.

FIG. 30.—Egg and ventral canal nucleus. ×440.

FIG. 31.—Endosperm cells and megaspore membrane; January 28. ×950.

FIG. 32.—Egg ready for fertilization; four-celled neck and jacket membrane; male cells, stalk, prothallial, and tube nuclei. ×440.

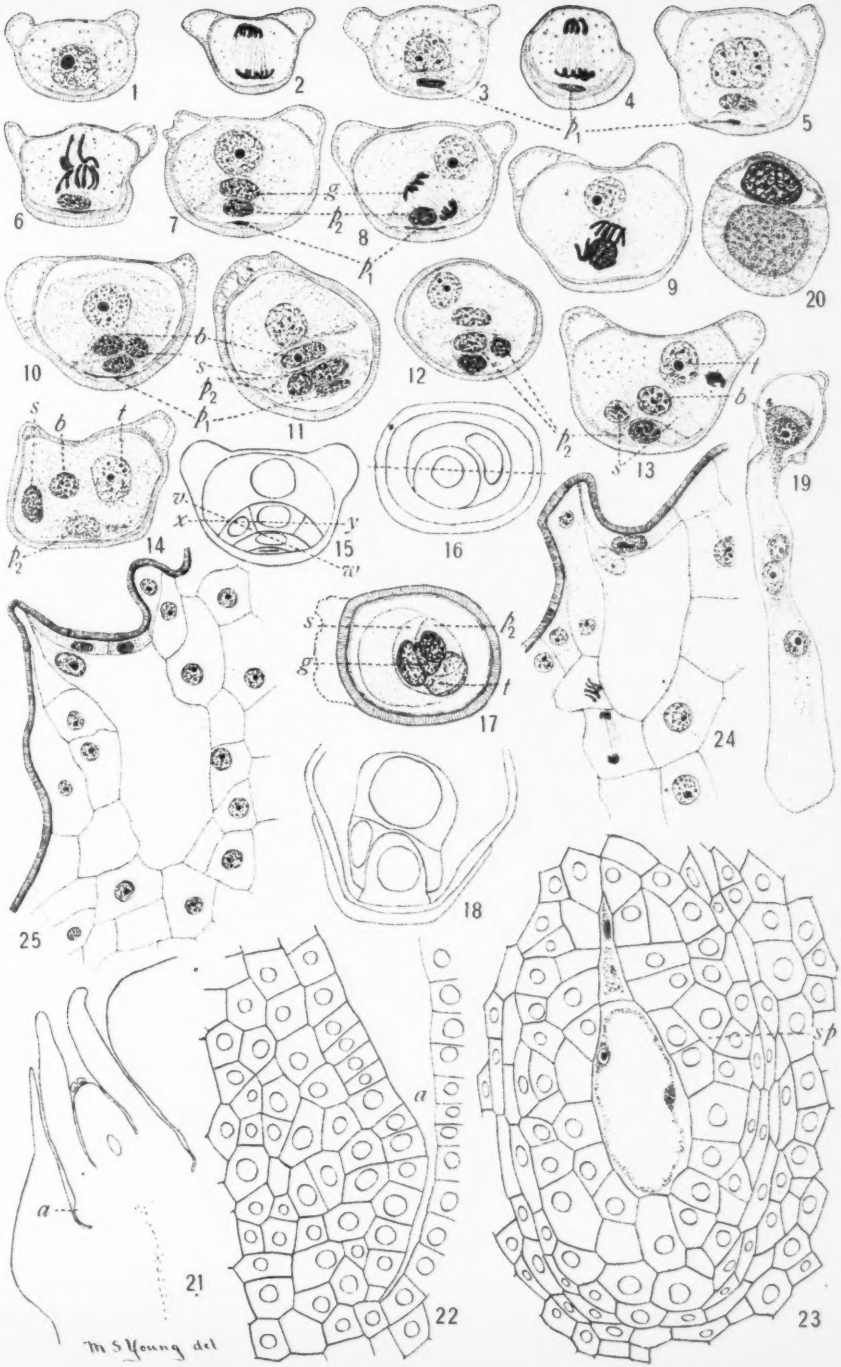
FIG. 33.—Effect of pollen tube on an archegonium; the membrane intact. ×440.

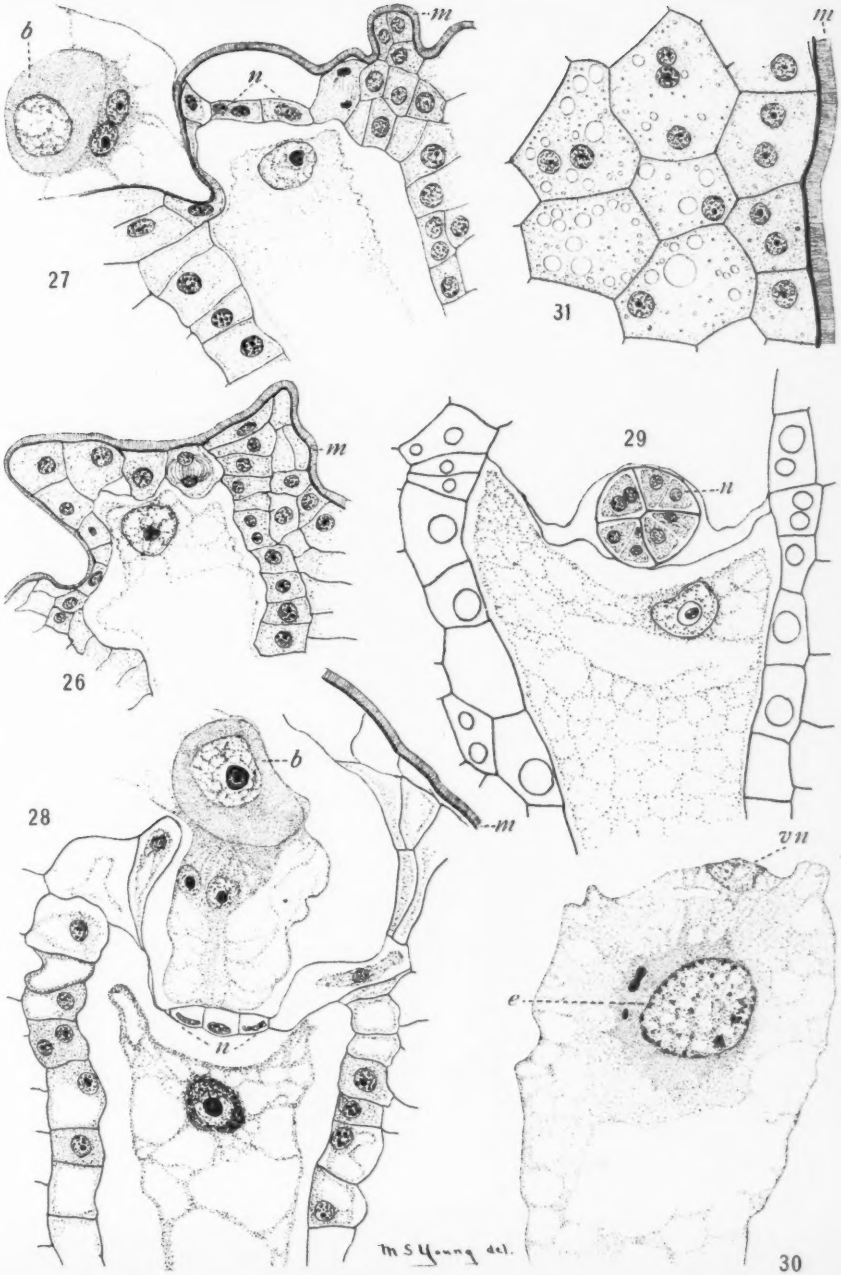
FIG. 34.—Two archegonia in a common jacket; two pollen tubes. ×340.

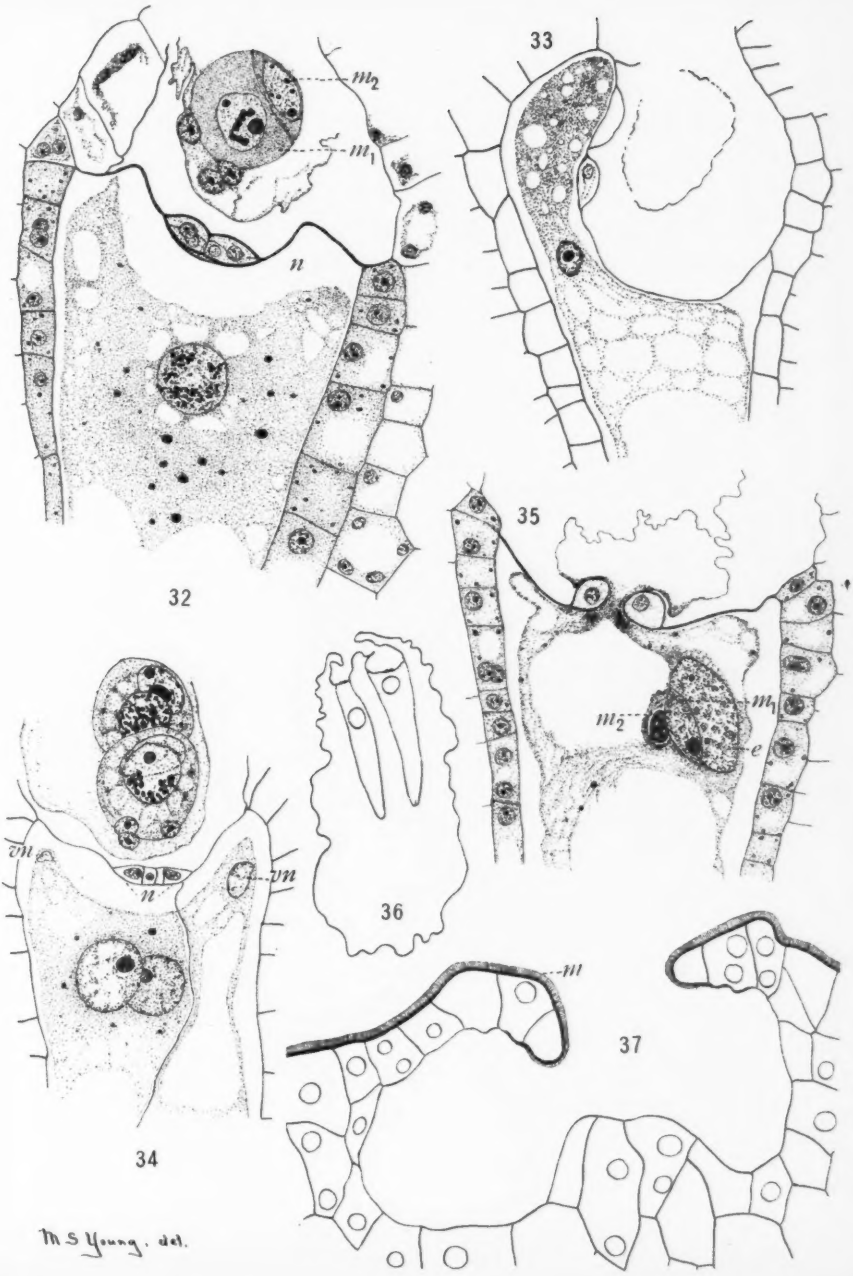
FIG. 35.—Fertilization. ×440.

FIG. 36.—Female gametophyte. ×45.

FIG. 37.—Megaspore membrane and archegonial cavities. ×440.


1
2
3
4
5
6
7
8
9
20
10
11
12
13
14
15
16
17
18
19
24
25
21
22
23
M S Young del
sp


b
n
m
27
m
31
m
26
29
n
b
28
m
n
vn
e
M S Young del.
30


m_2
m_1
n
32
33
35
m_2
m_1
e
vn
n
vn
34
36
m
37
M S Young. del.

THE ORIGIN OF RAY TRACHEIDS IN THE CONIFERAE

W. P. THOMPSON

(WITH SIXTEEN FIGURES)

Considerable attention has been devoted in recent years to the phylogenetic significance of ray tracheids in the Coniferae. PENHALLOW (1), arguing from their distribution and from his theory of their origin, concludes that the number in a given species is in direct proportion to its specialization, and that the forms where they are most numerous are derived from those where they are not so well developed. JEFFREY (2), on the other hand, from a study of certain traumatic phenomena, considers that the forms where they attain their greatest development, namely the pines, are the most ancestral, and have given rise by degeneration to the ones in which they occur sporadically. Apart from its intrinsic interest, it is hoped that the present study, by determining the origin of the ray tracheid, will supply a basis for its correct phylogenetic interpretation.

In carrying on the study, a thorough investigation was made of the character and mode of formation of the ray tracheid throughout the individual plant, but more especially in the primitive regions: seedling stem and root, young branch and young root of the adult, and the axis of the seed cone. The forms chosen for detailed investigation were our indigenous species of the hard and soft pines, *Pinus resinosa* and *P. Strobus*, but the results were confirmed in many other forms. The usual method was to follow a ray by means of a series of sections, from its beginning at the pith through the wood to the cambium.

The origin of the medullary ray at the pith has been described and figured by KNY (3) for *Pinus silvestris*. He states that in this region all the ray cells are parenchymatous, and elongated not radially but vertically. These long cells are often in connection with similar cells from rays lying above or below. Very soon they separate, shorten in the vertical direction, and elongate radially to form the typical medullary rays, which during their further course are separate. A later

writer, CONWENTZ (4), agrees with this description and extends it to include the root. I have found the observations of both writers to be practically true for *Pinus Strobus* and *P. resinosa*. In view of what is to follow, however, dissent must be expressed from the statement that during their further course the rays are separate. An important feature which neither writer emphasizes is that the rays in this young wood are low, rarely more than two cells in height.

The further course of the rays in root, stem, and cone axis shows essential differences; accordingly these regions are treated separately.

Root

Some distance from the pith certain peculiar tracheids appear, which KNY and other investigators seem to have overlooked.

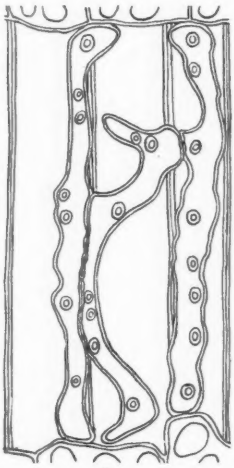


FIG. 1

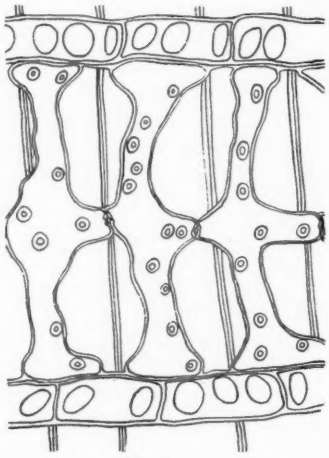


FIG. 2

FIGS. 1, 2.—Fig. 1, *P. resinosa:* young root; short tracheids extending between two rays; fig. 2, *P. Strobus:* young root; short tracheids with characteristic radial projections.

Examples of them are shown in figs. 1 and 2. They occur in radial rows, extending between two rays which lie in the same vertical plane. Consequently they are of various lengths, depending on the distance between the rays. Their form is also variable, often being quite uniform, like tracheids (fig. 1), often very irregular in outline.

A frequent peculiarity is the possession of arms (fig. 2) projecting radially either from the ends, or the middle, or from both, and meeting similar projections from their fellows. In this case the bodies of the cells are some distance apart. The walls of all these short tracheids are generally thinner, and their bordered pits much smaller than those of ordinary tracheids.

The usual sequence of these cells and their transformations toward the cambium are illustrated in fig. 3, which is a camera lucida drawing of two consecutive sections from a series of the root of *Pinus resinosa*. The cambium in this figure is toward the right (this orientation has been preserved throughout my illustrations). On their first appearance, toward the left of the figure, these tracheids have the more regular form approximating ordinary tracheids. Farther out they become irregular, usually with the above mentioned projections; then divisions occur, forming two superposed rows. These soon separate, taking up their position along the rays (center of figure) and forming irregular marginal cells, which gradually become regular and assume the form of true ray tracheids. In the ray at the bottom of the figure, ray tracheids have already been formed, but in one at the top this process has not yet been completed. In *Pinus Strobus* the sequence is similar. Often in both, the series is not so regular as the one just described; some stages may be hurried over, some greatly prolonged. In the one drawn, the transformation has taken place much more quickly than is usually the case, for the transitional stages may often be traced through several years' growth.

That these elements are indeed transitional is further shown in fig. 3 (*Pinus resinosa*) by the development on their walls of the dentations characteristic of the ray tracheids of the hard pines. When they first appear the elements are quite smooth, but as they assume more the shape and character of ray tracheids they acquire these dentations. One may often find intermediate cells whose walls are partly smooth like tracheids and partly dentate like ray tracheids (center of fig. 3).

It is evident that in the root of these forms we have a complete transition between short tracheids and ray tracheids; that by a process of division, of shortening, and of radial extension marginal ray tracheids have been produced from tracheary tissue.

The description so far has dealt with the marginal ray tracheids. The interspersed ones appear about the same time; their origin is illustrated in fig. 4. At the left of the figure are the irregular short tracheids extending between two rays. Farther to the left, that is, nearer the pith, these rays are more distant and the transitional tracheids are longer. To the right the rays gradually approach and

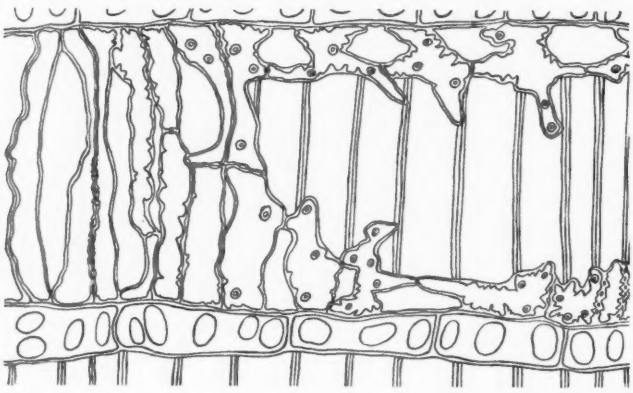


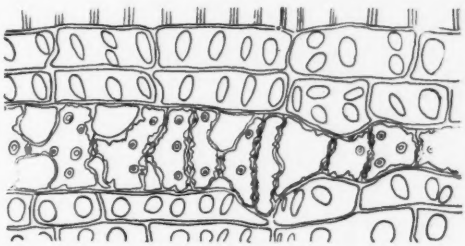
FIG. 3.—*P. resinosa:* young root; the transformation of short tracheids to marginal ray tracheids.

the tracheids shorten to form the regular interspersed ray tracheids which continue to the cambium. Thus interspersed ray tracheids originate from the same kind of tracheary element as the marginal ones. In the latter case the rays separate and the tracheids form along their margin. In the former, the rays draw together and the tracheids shorten and take their place in the center of the resulting composite ray.

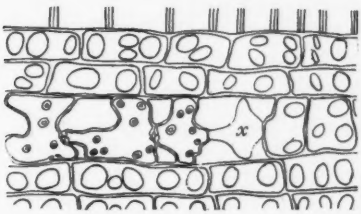
The formation of this ray calls attention to an undescribed phenomenon apparently rather common in *Pinus*, namely the fusion of rays. As noted above, when the rays originate at the pith they are usually only one or two cells in height; but in the adult wood many of them are much higher. In the root wood up to twelve years old, careful observations were made to determine to what extent the increased height was due to fusion. This was done by following the rays in a series of sections from the cambium to the pith. It was found that

practically every one of the high rays was formed by a combination of two or more lower ones. Only very rarely was a height of four cells attained otherwise. The so-called *primary* rays are then really formed *secondarily*, by fusion. To what extent this fusion is characteristic of the other forms has not yet been determined, but it would seem that the low ray is the primitive condition in the pines.

4



5



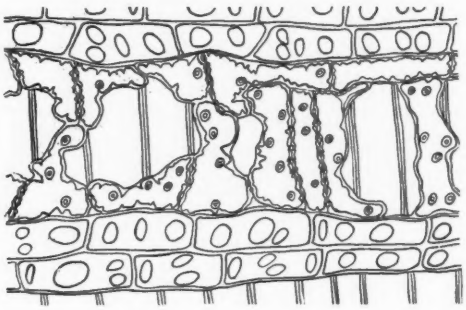
FIGS. 4, 5.—Fig. 4, *P. resinosa:* young root; the transformation of short tracheids to interspersed ray tracheids, and the fusion of rays; fig. 5, *P. Strobus:* young root; the replacement of ray tracheids by parenchyma; at *x* is a degenerating tracheid.

The approach of the rays does not always end in the production of one with interspersed tracheids. When the rays have drawn together till the intervening tracheids have formed true ray tracheids or are on the point of doing so, then these elements are often replaced by a row of parenchyma cells which soon assume the ordinary form and size. In fig. 5 at the left are the short transitional tracheids which are replaced at the right by high parenchyma cells. Farther toward the cambium these gradually become lower and indistinguishable from other cells of the ray.[1] Instances were also observed where the

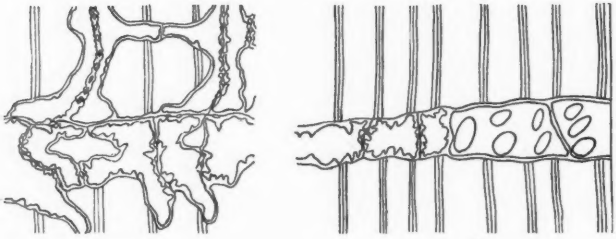
[1] In this drawing, and in the others as well, with the exception of figs. 8, 9, 14–16, the simple pitting of the wall of the parenchyma has been used to distinguish this tissue from the tracheary elements with their bordered pits, the nuclear and protoplasmic contents of the former being omitted.

fusion of rays eliminated a row of tracheids without replacement by parenchyma. The tracheids were simply "pinched" out, only the parenchyma cells continuing. In a few cases, even after true marginal ray tracheids had been formed as above indicated, the rays drew together and fused, with the result that the marginal ray tracheids were "pinched" out.

6



7



a *b*

FIGS. 6, 7.—Fig. 6, *P. resinosa:* young root; illustrating the formation of a double row of ray tracheids; fig. 7, *P. resinosa:* young root; formation of a wholly tracheidal ray, and its transformation into a parenchymatous one.

The origin of the marginal and interspersed ray tracheids and their relationship to the fusion of rays have been described. The more complicated case of the formation of two rows of tracheids on the margin of a ray is partly illustrated in fig. 6. At the left, toward the pith, are two rows of transitional tracheids touching end to end. Beyond the figure, nearer the medulla, these have been formed from a single row of longer tracheids. Toward the cambium the row on the

margin of the upper ray settles down to form regular ray tracheids while the other remains transitional. A repetition of the process in this row gives rise, beyond the figure, to a double series on the margin of the upper ray. In many cases, however, the second row is formed without the intervention of the transitional cells, this stage being either hurried over or completely omitted.

The only kind of ray tracheid whose formation has not yet been described is that composing the wholly tracheidal ray. When the rays are far apart, two or more divisions may take place in the long tracheids instead of the usual single one. Then one or more rows of transitional cells are formed midway between the rays, with other rows above and below, that is, touching the rays. When the latter rows separate from the central ones, these continue as irregular tracheidal cells which gradually become regular, forming a completely tracheidal ray. Fig. 7 represents only the ends of the series, a considerable space at the center being left out; in *a* are two rows of irregular tracheids, the lower of which settles down to form the completely tracheidal ray seen in *b*.

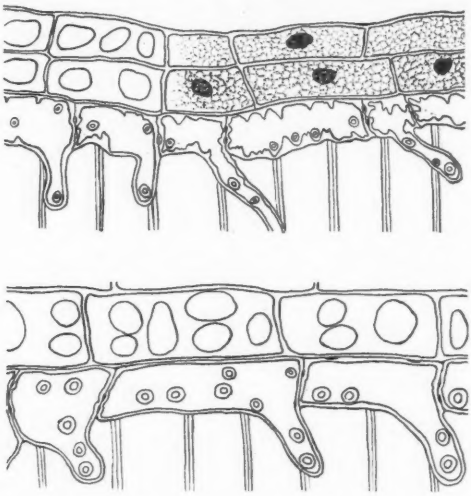
At the right in figure 7*b* another phenomenon is illustrated. This is the replacement of a tracheidal ray by a parenchymatous one. It is not an abnormality in this section, for numerous examples were seen. In fact it seems to be the common method of origin for secondary rays, that is for ones which do not run to the pith. Owing to the yearly increase in the circumference of the wood, many new parenchyma rays must be formed if the number in a given area is to remain at all constant, and it seems easier for them to be produced from cambial cells which give rise to ray tracheids than from longer ones which give rise to wood tracheids.

FIG. 8.—*P. resinosa:* young root; the development of a ray tracheid at the cambium.

In the development of some of the ray tracheids at the cambium further evidence is afforded of the origin of these structures from tracheary tissue. In fig. 8 the cambium is at the right. It has just given off a tracheid whose upper end is

"turning" along the ray and developing the buttresses characteristic of the ray tracheids of the hard pines. The lower part remains smooth. At this stage then, it is quite intermediate between a wood tracheid and a ray tracheid.

Farther from the cambium whole rows of tracheidal cells may be observed, each with a long tail-like projection extending from one



FIGS. 9, 10.—Fig. 9, *P. resinosa:* young root; a row of ray tracheids with tail-like projections; fig. 10, *P. Strobus:* adult root; ray tracheids with "tails."

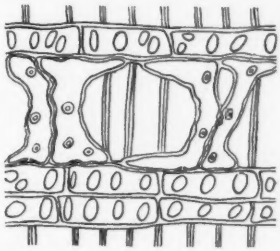
end (fig. 9), and this end is always the one nearer the cambium. The "tail" is evidently the smooth part of the tracheid as seen at the cambium. Moreover, in *Pinus resinosa* the "tail" lacks the characteristic dentations present on the rest of the cell. Apparently then it is the result of an incomplete shortening of the tracheid. Fig. 10, from an old root of *Pinus Strobus,* shows that these projections persist in the mature wood, but, as is to be expected, they are neither so numerous nor so conspicuous.

Stem

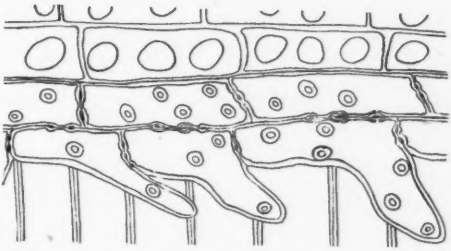
The root is admittedly more conservative than the stem. Accordingly in the latter the evolutionary processes are not so well repre-

sented. There is a hurrying over the early stages, so that an actual series can rarely be observed. Nevertheless, the transitional form of tracheid is occasionally found, and has been drawn in fig. 11 from the second year's growth of *Pinus Strobus*. Tail-like projections are quite common, and have been illustrated in fig. 12.

11



12



FIGS. 11, 12.—Fig. 11, *P. Strobus:* young stem; transitional tracheids; fig. 12 *P. Strobus*: adult stem; ray tracheids with "tails."

The only good stem series showing the transformation from tracheids to ray tracheids was observed in old stem wood of *Pinus resinosa* which had been wounded (fig. 13). The rapidly shortening series of tracheids ends at the right in true ray tracheids with buttressed walls. In this wounded material also the tail-like projections were very numerous and very large. Both features are to be accounted for as traumatic reversions similar to those described by JEFFREY for *Cunninghamia* (**2**), in which form though ray tracheids are not normally present they were found in connection with a wound.

Even in the normal adult ray tracheid, however, we find indications of its tracheary origin. One of these is the occurrence of tertiary

spirals. This has been described by BAILEY (**5**) for some species of *Pinus* as wall as for *Picea* and *Pseudotsuga*. He states: "Ray tracheids appear to follow closely the wood tracheids. Where spirals

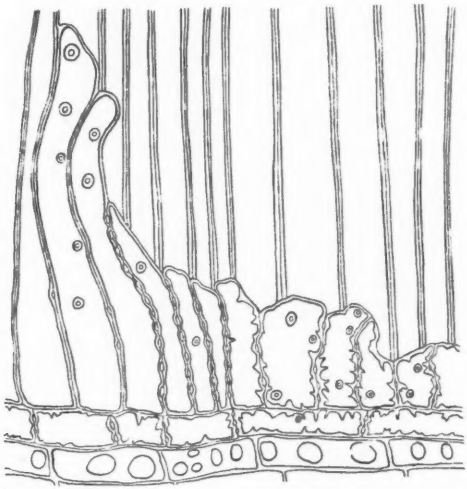


FIG. 13.—*P. resinosa:* adult stem; a series of tracheids from a wounded region, showing transformation to ray tracheids.

are strongly developed (summer wood) in the latter elements, they will also appear in the former." This concomitant occurrence of tertiary spirals on the elements in question argues community of origin.

Seedling

So far the study of the seedling has presented no features not observed in the corresponding regions of the adult, except that here the ray tracheids are later in appearing.

Cone axis

True ray tracheids are quite absent from the cone axis, as JEFFREY and CHRYSLER (**6**) have pointed out. I have found their place taken, however, by the bent ends of the tracheids. Fig. 14 shows a tracheid

whose end is bent along a ray for a remarkable distance. Such tracheids are very common in the cone axis, more than half the rays showing at least one in some part of their short course. Evidently they do the work of the ray tracheid, and probably represent the first step in a process of turning and shortening which, as described above, ends in the production of ray tracheids. Similar bent ends are sometimes to be found in the young stem, and are evidently to be interpreted in the same way.

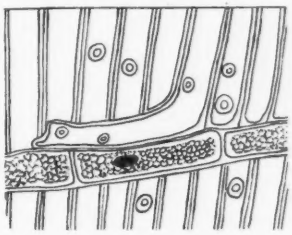


FIG. 14.—*P. Strobus:* cone axis: the end of a tracheid bent along a ray.

Relation to albuminous cells

In the light of what has been said of the origin of ray tracheids, an interesting circumstance is furnished by their relationship to the albuminous cells of the ray. STRASBURGER (**7**) points out the homology between the latter and sieve tubes. "The albuminous cells are higher than the ordinary cells of the ray and like sieve tubes have sieve areas, soon lose their contents, and ultimately collapse." They are then virtually *ray sieve tubes*. Now in the Abietineae, where ray tracheids are numerous, albuminous cells were always found conterminous with them through the cambial region. This is illustrated in fig. 15 even for a row of interspersed ones. In the Araucarineae and Taxaceae, where there are no ray tracheids, there are no albuminous cells. In those Taxodineae and Cupressineae where no ray tracheids were seen, no albuminous cells were found. And in *Thuja*, where ray tracheids occur sporadically, the only albuminous cells were those lining up with ray tracheids. Albuminous cells are then always associated with ray tracheids (an interesting exception is noted below). This association and their sieve tube character indicate that they bear the same relationship to ray tracheids as the sieve tubes to wood tracheids. They thus afford valuable collateral evidence of the tracheary origin of their representatives in the wood.

The exception referred to is found in *Abies balsamea*. Here almost every ray passing through the bast has albuminous cells on its margin,

but very few have ray tracheids. The albuminous cells are never in line with the parenchyma cells of the ray, but always above or below them. Often they are in line with two or three degenerating cells on the wood side. These facts support the view of JEFFREY (**2**) that the scarcity of ray tracheids in certain Abietineae, including *Abies*, is the result of degeneration. The albuminous cells, elsewhere inseparable companions of the ray tracheids, persist, while the ray tracheids themselves have disappeared. The latter are probably represented by the degenerating cells mentioned.

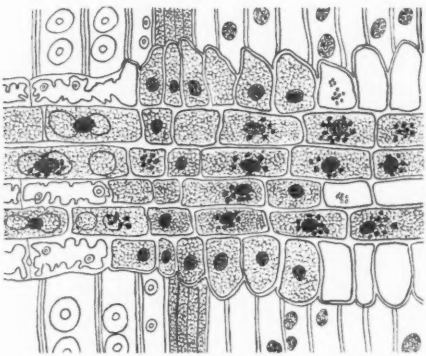


FIG. 15.—*P. resinosa:* old stem; cambial region showing albuminous cells in line with ray tracheids.

Another observation supporting this view is that of the occurrence of ray tracheids in the wounded root of *Abies amabilis*. Hitherto all observers have reported ray tracheids absent from the wood of this species. Yet in a piece of root wood which had been wounded several times, undoubted ray tracheids were developed in considerable numbers. This phenomenon parallels the traumatic revival of ray tracheids in *Cunninghamia* described by JEFFREY (**2**), and admits of a similar interpretation, in this case their ancestral presence in the genus *Abies*.

General considerations

The character and relationships of the ray tracheid have been described in the various regions of the individual plant. As has been shown, it is in the young root that the proof of its origin from tracheary tissue is most conclusive. Here transitions were observed from short tracheids extending between the rays to ray tracheids of all kinds. A significant feature, which has not yet been emphasized in connection with these transitional areas, is the occurrence of degenerating cells. One of these has been incidentally illustrated in fig. 5 at *x*. They

are structureless, shadowy outlines replacing transitional tracheids, which they resemble in form, although often somewhat more irregular. They occur in considerable numbers wherever the former elements are found, and are evidently to be regarded as "degeneration products," to which transitional structures are always subject. As such they emphasize the transitional character of the regions in question, and help to complete the chain of evidence for the origin of the ray tracheids from tracheary tissue.

PENHALLOW'S argument (1) for his theory that ray tracheids are derived by modification from the parenchymatous cells of the ray, is the occurrence of ray tracheids conterminous with parenchyma cells. But this is so rare that DEBARY (8) was led to assert that it never occurred. I must confirm PENHALLOW'S observation, however, especially in the young plant, where such an appearance is more common than in the adult. I have observed and figured this feature in the formation of secondary parenchymatous rays from wholly tracheidal ones (fig. 7), and in the replacement of interspersed ray tracheids by parenchyma (fig. 5). I have observed also a similar replacement of marginal ray tracheids by parenchyma cells. In these instances, however, it is to be noted that the parenchymatous cells are toward the cambium (to the right in the figures), and so *replace tracheary elements*, the reverse of what is required from PENHALLOW'S point of view. This is indicated in the very figure which PENHALLOW uses to illustrate the fact that ray tracheids may abut on parenchyma cells (his figure is reproduced herewith, fig. 16). The direction in which the oblique end walls are inclined, and in which the tail-like projection points, which is seen on one of the ray tracheids on the top of the ray, indicates, as shown above (figs. 8–10, 12), that the cambium is to the left of his figure. The parenchyma cell which is in line

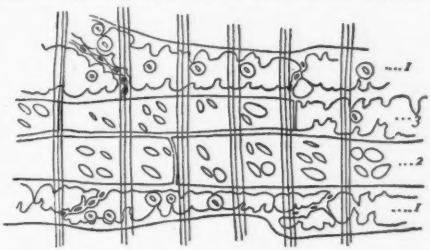


FIG. 16.—*P. serotina:* medullary ray showing (1) the ray tracheids with dentate walls, (2) the structure of the parenchyma cells, (3) tracheids conterminous with parenchyma cells.—From PENHALLOW (1).

with the ray tracheid is then nearer the cambium, and so what we really have is the replacement of a row of tracheary elements by parenchymatous ones. The origin of such a row of ray tracheids is to be looked for toward the pith, and, as I have shown above, the ray tracheids arise here either in connection with transitional tracheids, or, when these are omitted, above or below the ray and not in line with parenchyma cells.

The intimate study of the medullary ray from its beginning at the pith in the different regions of the individual plant, besides disclosing the origin of the various types of ray tracheid, has drawn attention to important features in the distribution of these structures. KNY has called attention to their absence in the first year's growth of the stem, and JEFFREY and CHRYSLER state that they are not present in the seed cone. In the young branch of *Pinus Strobus* they appear sporadically during the second and third years, and then increase slowly in number until the adult condition is attained after about ten years. In the seedling they are still later in appearing, as is also the case in the young root. In the latter region they never become so numerous as in the stem. In *Pinus resinosa* they appear in abundance much earlier and increase in number much more rapidly, reaching the adult condition in five or six years. They are never found in the cone axis of either species. Thus in the primitive regions of the plant ray tracheids do not occur, and therefore they must be regarded as specializations. That they are of cenogenetic origin is further indicated by the fact that in the older pines, the Pityoxyla of the Cretaceous, as described by JEFFREY and CHRYSLER (**6**), no ray tracheids occur.

In view of their origin and distribution, ray tracheids are regarded as specialized structures and their phylogenetic meaning so interpreted. Those woods in which they are most abundant are considered most modern, unless, as in *Abies*, it can be shown that they have been secondarily lost. Their character when present is as important as their number. For example, if the early growth shows many transitional elements or a large development of tail-like projections, then the wood is stamped as primitive. Again, the smooth-wall form in the soft pine is more tracheid-like and therefore more primitive than the dentate form of the hard pine, an inference which

is strengthened by the earlier and more rapid development of ray tracheids in *Pinus resinosa* than in *Pinus Strobus*.

Summary

1. In the young root complete transitions may be observed from short tracheids extending between the rays, to ray tracheids both marginal and interspersed. In the young stem only remnants of the transition usually remain. The complete series, however, may occur traumatically.

2. Further evidence of the origin of ray tracheids from tracheary tissue is found in (1) their development in the cambial region of the young plant, (2) the occurrence of tail-like projections, and (3) the possession of tertiary spirals.

3. The occurrence of ray tracheids bears a definite relation to that of albuminous cells.

4. In *Abies* the possession of albuminous cells and the traumatic occurrence of ray tracheids indicate that the latter are vestigial.

5. The regional and fossil distribution of ray tracheids indicates their ancestral absence in the pines.

6. The hard pines are more specialized than soft ones.

7. The large rays of *Pinus* are usually formed by the fusion of smaller ones.

8. Ray tracheids are often replaced by parenchyma cells. The importance of this in the formation of secondary parenchymatous rays has been indicated.

This study was undertaken at the suggestion of Mr. R. B. THOMSON and carried on with his constant advice. My warmest thanks are due to him for quite exceptional kindness throughout the course of the work.

UNIVERSITY OF TORONTO

LITERATURE CITED

1. PENHALLOW, D. P., North American Gymnosperms, 1907.
2. JEFFREY, E. C., Traumatic ray tracheids in *Cunninghamia sinensis*. Annals of Botany **22**:593–602. *pl. 31*. 1908.
3. KNY, L., Anatomie des Holzes von *Pinus silvestris*. Berlin. 1884.

4. CONWENTZ, H., Monographie der Baltischen Bernsteinbaume. Danzig. 1890.
5. BAILEY, I. W., The structure of the wood in the Pineae. BOT. GAZETTE **48**:47–55. *pl.* 5. 1909.
6. JEFFREY, E. C., and CHRYSLER, M. A., On cretaceous Pityoxyla. BOT. GAZETTE **42**:1–15. 1906.
7. STRASBURGER, E., Lehrbuch der Botanik. Jena. 1908.
8. DEBARY, A., Comparative anatomy of phanerogams and ferns. English edition. Oxford. 1884.

ON THE RELATIONSHIP BETWEEN THE LENGTH OF THE POD AND FERTILITY AND FECUNDITY IN CERCIS

J. ARTHUR HARRIS

(WITH ONE FIGURE)

It is well known to botanists that the egg cell is not the only structure affected by fertilization. GOEBEL points out[1] that in the Hepaticae an accompanying result is often seen in the production of a further development of the envelopes of the ripening sporangium. Again, in the same work he suggests[2] that the stimulus exercised by pollination in the flowering plants is most probably chemical. The preparation of the ovules for fertilization is dependent in some cases upon pollination. For instance, in such plants as *Corylus*, *Alnus*, *Quercus*, and some of their allies, there is no sign of the placenta in the ovary, to say nothing of ovules, at the time of pollination. In most species of Orchidaceae the ovules are laid down at the time of pollination, but still are rudimentary. The stimulus exercised by the pollen tube induces the further development of the female sexual apparatus in these plants.

PFEFFER[3] joins with GOEBEL in considering that the penetration of the pollen tube may serve as a stimulus to the development of the ovary, and cites the seedless fruits studied by MÜLLER-THURGAU and the observations on *Ficus* by TREUB.

JOST[4] writes: "The germination of the pollen tube has an exciting influence on the development of the fruit. This is particularly noticeable in certain cultivated plants, which, as for example currants and sultana raisins, produce no seeds, the ovules having degenerated. If the stigmas of these plants be not pollinated, the fruit fails to develop, but pollination leads to development without leading to any fertilization."

[1] Organography of plants **2**:105. 1902.

[2] *Op. cit.* **1**:269–270. 1900.

[3] Physiology of plants (English transl.) **2**:173. 1900–1906.

[4] Lectures on plant physiology (English transl.) p. 370. 1907.

This is not the place for a review of the literature bearing upon these interesting chemical, physiological, and morphogenetic problems, however one wishes to designate them. The views of the three botanists just cited are sufficient to show the interest which is being taken in these problems. Several writers have contributed to the literature. Without any attempt at arrangement for priority or extent of investigation, I mention EWART (**1, 2**), SOLACOLU (**7**), TREUB (**8**), MÜLLER-THURGAU (**5**), NOLL (**6**), and FITTING (**3**). FITTING (**4**) has recently given a review, with a bibliography, of the chief literature in connection with an account of his own work on the Orchidaceae. From his experimental studies in this family he is led to the conclusion that the stimuli involved in the ontogeny of the fruit are in part due to an organic substance, not an enzyme, external to the pollen grain, in part to the growth of the pollen tubes, and finally in part to the development of the fertilized ovules.

The observations of the authors mentioned above deal chiefly with the influence of pollination as a stimulus inducing the development of the ovary up to a stage where the fertilization of the ovules is possible, or a little beyond. These phases of the problem are much more easily studied experimentally than that of the influence of the developing ovule upon the growth of the ovary. The present investigation bears upon this point.

If the developing seed excretes some substance which acts as a stimulus to the development of the ovary wall, or in some other manner exerts an influence upon it, it seems not unreasonable to suppose that the effect would be greater if several seeds were developing than if there were only one or a very few.

Our problem is essentially this: Does the number of ovules which develop to matured seeds influence the size of the fruit, and to what extent?

In all of the pods which have matured at least one seed, the stimulus to development due solely to the penetration of the pollen tube (as distinguished from the possible influence of the developing zygote) should be the same for all pods, unless the quantity of pollen tubes which penetrate the tissue of the style differs from ovary to ovary, and the intensity of the stimulus is to some extent proportional to their number. The problem is surrounded by a good many difficul-

ties. There seem to be at least four ways in which a relationship between the number of seeds developing and the size of the fruit might arise.

a. Fruits with a larger number of ovules are apt to be larger, if for no other reason, simply because the placental space required is greater. In the long run fruits with larger numbers of ovules also have larger numbers of seeds,[5] and an influence on fruit length at first attributed directly to the development of the seeds might be due indirectly to the number of ovules.

b. The space required for the matured seeds might, through the purely mechanical effects of crowding, result in the fruits with greater numbers of seeds being larger in size.

c. The developing seed might by means of some excreted product, or in some manner not yet suggested, directly induce a greater development of the ovary.

d. Both the number of ovules developing into seeds and the size attained by the fruit may be to some measure dependent upon some other character; say, for example, the position of the fruit on the inflorescence axis, or the number of fruits developing per inflorescence. The correlation between them might then be due merely to their mutual dependence upon some other character.

In the literature one finds only a few references to the relationship between the fertility of a fruit and its size. EWART (1) gives tables showing numbers of seeds and mean weight of fruit for three series of 125, 48, and 134 fruits of one variety of apples, which indicate that weight increases with number of seeds. According to EWART, MÜLLER-THURGAU found that in apples and pears the size of the fruit and the number of seeds are interdependent. MÜLLER-THURGAU cut off four of the five stigmas in the pear blossom, fertilized the remaining one, and thus produced asymmetrical fruit. EWART concludes: "Es steht demnach ohne zweifel fest, dass den Kern einen Wachtumsreiz auf die zur Fruchtbildung bestimmten Gewebe ausübt."

In view of the four possibilities suggested above, I think it is quite evident that great caution should be used in asserting that the number of ovules developing into seeds has *per se* any influence upon

[5] This statement is based on the results of many series of unpublished observations.

the size of the fruit. I believe that in such cases the analytical methods of modern higher statistics applied to large bodies of data are fitted to give results of real value.

The purpose of the present paper is to present the results of an attempt to measure the intensity of the interrelationship between the length of the pod and (*a*) the number of ovules formed, (*b*) the number of seeds developing, and (*c*) the fecundity, that is, the ratio of number of seeds developing to number of ovules formed per pod, in *Cercis canadensis*.

As material for a first study, the number of ovules formed and the number of seeds developing per pod were counted and the length measured in 3,000 pods, collected at Meramec Highlands, St. Louis, Missouri, in the autumn of 1905. To secure as representative material as possible, 50 pods from each of 60 individuals were taken. The measurements of pod length were taken to the nearest millimeter, and then grouped in classes of three millimeter range for purposes of calculation.

TABLE I

FERTILITY AND LENGTH OF POD FOR 3,000 *Cercis* FRUITS

	45–47	48–50	51–53	54–56	57–59	60–62	63–65	66–68	69–71	72–74	75–77	78–80	81–83	84–86	87–89	90–92	93–95	96–98	99–101	102–104	
1	..	..	..	1	..	..	...	...	...	...	...	...	...	...	..	..	..	.	..	..	1
2	..	..	1	2	1	7	4	4	1	...	...	...	...	1	..	..	..	..	..	..	21
3	2	1	1	3	3	2	...	1	...	...	...	...	...	...	..	..	..	..	..	..	13
4	1	1	1	3	6	8	9	8	3	5	2	1	1	...	..	..	..	..	..	..	49
5	..	..	1	1	8	10	20	24	27	18	14	4	1	1	..	..	..	..	..	..	129
6	..	..	..	..	1	1	2	...	...	...	...	...	...	...	..	..	..	..	..	..	4
7	..	..	..	3	9	14	22	16	12	11	7	4	2	1	..	..	..	..	..	..	101
8	..	..	2	11	9	37	72	62	56	57	57	24	8	4	..	..	..	..	..	..	399
9	1	..	..	2	4	23	48	78	94	102	99	60	40	13	5	..	..	..	..	..	569
10	..	..	..	..	..	..	1	...	...	...	...	...	...	...	..	..	..	..	..	..	1
11	..	..	1	..	1	5	2	1	2	1	1	2	2	1	..	..	..	..	..	..	19
12	..	..	1	2	3	14	18	21	20	16	22	10	7	2	..	1	..	1	..	..	138
13	..	..	1	1	3	13	22	54	67	70	74	48	33	19	3	8	1	2	1	..	420
14	..	..	..	..	2	5	17	30	58	115	115	96	59	48	20	11	..	..	1	..	577
15	..	..	..	1	..	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..	3
16	..	..	..	..	..	1	3	3	2	4	2	2	..	..	..	..	..	..	1	..	18
17	..	..	..	..	1	2	7	4	10	22	16	10	6	2	1	2	..	..	..	..	83
18	..	..	..	..	..	4	3	6	16	23	36	39	29	16	5	6	..	1	..	1	185
19	..	..	..	..	..	..	4	3	19	22	38	45	38	25	10	7	4	3	4	2	224
20	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1
21	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0
22	..	..	..	..	..	1	...	...	1	1	1	3	1	..	..	..	..	..	..	..	8
23	..	..	..	..	..	..	1	...	1	1	3	2	2	3	2	2	..	..	..	..	17
24	..	..	..	..	..	..	..	..	2	5	...	5	3	1	1	2	1	..	..	..	20
	4	2	9	30	51	147	255	315	391	474	487	357	232	137	47	39	6	7	7	3	3000

Table I gives the data. In the first column to the left the number of ovules formed and the number of seeds developing per pod are

shown in the form of fractions, in which the number of ovules is given as the denominator and the number of seeds developing as the numerator. For each of the twenty-four seed-ovule classes found in our material, the frequencies of the different pod lengths are tabulated out.

The physical constants describing these characters are:

Average ovules	4.6947 ± 0.0111
Standard deviation of ovules	0.9041 ± 0.0079
Coefficient of variation of ovules	19.258
Average seeds	4.0613 ± 0.0134
Standard deviation of seeds	1.0849 ± 0.0094
Coefficient of variation of seeds	26.712
Average length of pod	76.181 ± 0.095
Standard deviation of length of pod[6]	7.6814 ± 0.0669
Coefficient of variation of length of pod	10.496
Average index, seeds/ovules	0.8650 ± 0.0020
Standard deviation of index	0.1587 ± 0.0014

These constants require no discussion here; they enable us to pass to the determination of the degree of interdependence of the fertility characters and the length of the fruit.

Consider first the correlation between number of ovules per pod and the length of the pod. We find $r_{ol} = 0.4278 \pm 0.0101$. Remembering that correlation is measured on a scale of 0 to ± 1, we see (*a*) that the sign of the relationship is positive, that is, that as the number of ovules per pod increases the length also increases, and (*b*) that the relationship is a moderately close one.

The degree of interdependence between the two characters may be made clearer by expressing it in terms of regression instead of correlation. The equation to the regression straight line is

$$y = 56.119 + 3.634x.$$

In this case y = length of pod and x = number of ovules per pod. From this equation we see that a pod having an ovule more than the average of the population would be 3.6 mm. longer than the average length.

[6] SHEPPARD'S correction was applied to the second moment in the calculation of the constants for length of pod. It was not used for the fertility characters, where we are dealing with integral variates, nor in the seed-ovule indices.

The regression line and the empirical mean length for the different numbers of ovules per pod are shown in a diagram (fig. 1). To the eye the agreement is very good, except at the upper extreme, where the observations are relatively few. We may obtain some idea of

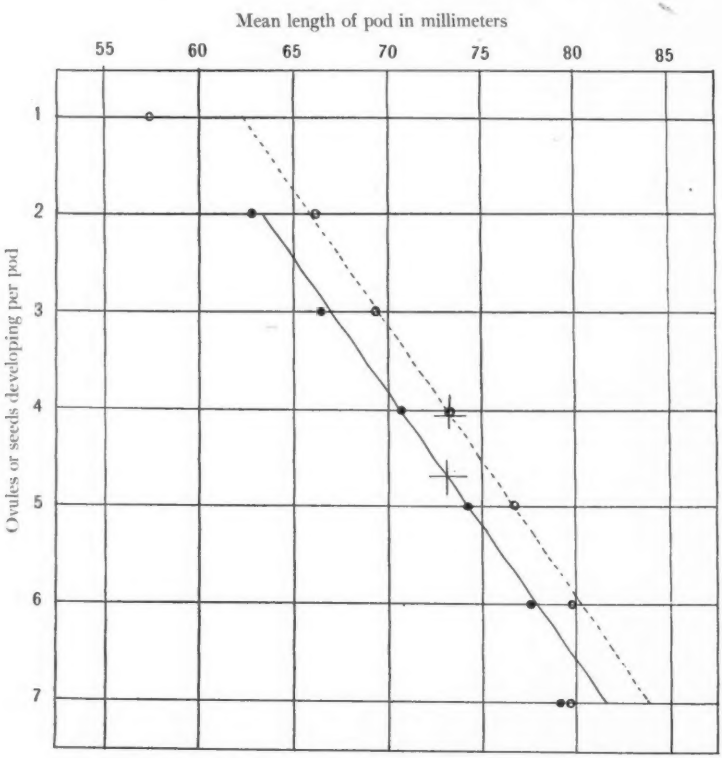


FIG. 1.—Diagram of slope of regression: straight lines, length on ovules and on seeds; solid line, theoretical length of pod for ovules; dotted line, theoretical length of pod for seeds; solid dots, observed length for ovules; circles, observed average length for seeds.

the amount of discrepancy between the theoretical line and empirical means by taking the average deviation on both sides of the line given by the equation. Considering the weight to be attached to a deviation to be proportional to the number of observations on which it is based,

we find average deviation = 0.248 mm. A discrepancy of a fourth of a millimeter in a character such as the pod length of *Cercis* may seem to most biologists quite negligible, but I rather suspect that the falling off in mean pod length in pods with seven ovules is significant. The point is of little practical importance for our present discussion.

For number of seeds maturing per pod and length of pod we find the interdependence $r_{sl} = 0.5055 \pm 0.0092$, and the equation to the regression straight line

$$y = 58.645 + 3.579x,$$

where the significance of y is as above, and x = seeds per pod. The diagram (fig. 1) shows the agreement between the line given by the equation and the empirical means. Throughout the central region of the distribution of the number of seeds developing, the fit is very good, but at both ends the observed means fall considerably below those to be expected from the equation. If these deviations are biologically significant, and not due merely to the small number of observations which fall at the extremes of the range, they indicate that both the pods developing only a single seed and those developing the maximum number of seeds are somewhat dwarfed in length as compared with the whole series of 3,000 pods. I am not yet ready to discuss the reasons for this condition.

Comparing the constant for ovules and length, and seeds and length, we find

For ovules and length, $r = 0.4278 \pm 0.0101$
For seeds and length, $r = 0.5055 \pm 0.0092$

Difference = 0.0777

Since the correlation is actually, though only slightly, higher for seeds developing than it is for number of ovules formed, it would appear that the number of seeds developing must have some connection with the length of the pod independent of the interdependence for length and ovules.

As pointed out early in this paper, one of the difficulties in asserting that there is a real physiological relationship between the number of seeds developing and the size of the fruit arises from the fact that

the correlation between the number of ovules formed and the size of the fruit may be of such a magnitude that it is impossible to tell without relatively refined statistical analysis whether the influence apparently due directly to the seeds may not be referred to the ovules. In the present case, for instance, the correlation for number of seeds developing exceeds only very slightly that for number of ovules formed.

We may determine whether the number of seeds developing has *per se* any correlation with the length of the pod by the following simple process. We sort our material into classes according to the number of ovules per pod, and then into subgroups according to the number of seeds developing, and determine the mean pod length for each of these subgroups. If now the length of the pod and the number of seeds developing are in some measure interdependent, we should expect to find significantly different mean lengths for pods of the same number of ovules, but different numbers of matured seeds.

TABLE II

MEAN LENGTH OF PODS FOR DIFFERENT NUMBERS OF SEEDS PER POD IN THE FOUR CHIEF OVULE CLASSES

NUMBER OF SEEDS PER POD	NUMBER OF OVULES PER POD			
	3	4	5	6
1.....	55.46	61.75	64.00	.....
2.....	64.24	66.94	68.73	69.00
3.....	68.37	69.06	70.37	72.00
4.....	.....	72.41	73.85	73.97
5.....	.....	.....	76.39	77.72
6.....	.....	.....	.....	79.76

Such an arrangement of the data is given in table II for the classes in which the number of observations is sufficiently large to give smooth results. Here the results are very clearly favorable to the hypothesis of a relationship between the number of seeds developing and the length of the pod, independent of the relationship for number of ovules, for the mean increases as the number of seeds increases when we work with constant numbers of ovules per pod.

The same problem may be approached in another manner, that has the advantage of giving a terse quantitative statement of the independent relationship between the number of seeds developing

and the length of the pod. The partial correlation coefficient gives the correlation between two characters, say seeds (s) and length (l) for constant values of a third character, number of ovules (o).

A necessary preliminary is to determine the correlation between number of ovules per pod and number of seeds developing per pod. We find $r_{os}=0.7297\pm0.0058$. Clearly with such a large value for the correlation between ovules and seeds we would expect some relationship between the number of seeds developing and the length of the pod, having no direct physiological significance whatever, but due merely to the fact that since number of ovules and number of seeds are closely correlated, and number of ovules and length of pod are correlated, number of seeds and length of pod *must also be correlated*. It is the influence of the ovules which we wish to remove by means of the partial correlation coefficient. The familiar formula is

$$\rho_{sl}=\frac{r_{sl}-r_{os}r_{ol}}{\sqrt{1-r_{os}^2}\sqrt{1-r_{ol}^2}},$$

which gives

$$\rho_{sl}=0.3128\pm0.0111.^{7}$$

I think this is a rather significant result. It not only shows that there is a physiological or morphogenetic relationship between the number of seeds developing and the length of the fruit independent of the correlation for ovules and length, but tells us the intensity of the interdependence as well.

There is still another way in which the influence of the ovules may be, to some extent at least, cleared away. Instead of correlating between the actual number of seeds maturing per pod and the length of the pod, the correlation between the relative number of seeds developing per pod (that is, the ratio or index seeds/ovules per pod) and the length of the pod may be found: $r_{il}=0.2906\pm0.0113$. This constant indicates very clearly that there is a real interdependence of number of seeds developing and fruit length, which is independent of the correlation for number of ovules and length of fruit.

7 The probable error of ρ_{sl} is from the formula $E\rho_{sl}=0.67449\,.1-\rho_{sl}/\sqrt{n}$. Mr. DAVID HERON, of University College, London, tells me that he has recently demonstrated the correctness of this formula and has the proof in press.

31913

Comparing the two methods of obtaining the independent correlation between number of seeds and length we have:

Correlation of index and length	0.2906 ± 0.0113
Partial correlation coefficient	0.3128 ± 0.0111
Difference	0.0222

This difference is certainly of no practical importance.

Summary

1. In considering the influence of the number of seeds developing upon the dimensions attained by the fruit, the number of ovules formed cannot be disregarded, since a correlation attributed directly to the influence of the development of the seeds may be in part at least due to an interdependence between the number of ovules formed and the dimensions of the fruit. The influence of the number of ovules can be neglected only when the coefficient of correlation between number of ovules and size of fruit is demonstrated to be zero. This point is well illustrated by a series of 3,000 pods of *Cercis*, where the correlations for ovules and length is $r_{ol}=0.428 \pm 0.010$; while the correlation for seeds per pod and length is only $r_{sl}=0.506 \pm 0.009$.

2. Two methods for freeing the correlation between the number of seeds developing and the length of the fruit from the influence of the relationship between the number of ovules formed and the length of the pod are suggested; the first is the determination of the partial correlation coefficient, that is, the correlation between number of seeds and length for constant values of numbers of ovules per pod; the second is the determination of the correlation between the index $\frac{\text{Seeds developing per pod}}{\text{Ovules formed per pod}}$ and the length of the pod.

The results from the data in hand are in close agreement,

$$\rho_{sl}=0.313 \pm 0.011$$
$$r_{il}=0.291 \pm 0.011$$
$$\rho_{sl}-r_{il}=0.022$$

and we conclude that of the gross correlation of about $\rho=0.500$ for number of seeds and length of pod a considerable portion, say $\rho=0.300$ roughly, is due to some morphogenetic or physiological relationship between the number of seeds developing and the length of the pod.

LITERATURE CITED

1. EWART, R., Blütenbiologie und Tragbarkeit unserer Obstbäume. Landwirtschaftl. Jahrb. **35**: 1906.
2. ———, Die Parthenocarpie oder Jungfernfrüchtigkeit unserer Obstbäume. Berlin. 1907.
3. FITTING, H., Die Beeinflussung der Orchideenblüten durch die Bestäubung und durch andere Umstände. Zeitschr. Botanik. **1**: 1909.
4. ———, Entwickelungsphysiologische Probleme der Fruchtbildung. Biol. Centralbl. **29**: 193–206, 225–239. 1909.
5. MÜLLER-THURGAU, H., Kernlose Traubenbeeren und Obstfrüchte. Landwirtsch. Jahrb. Schweiz. 1908.
6. MOLL, F., Ueber Fruchtbildung ohne vorausgegangene Bestäubung (Parthenocarpie) bie der Gurke. Sitzungsber. Niederrh. Geo. Nat. Heilkunde. Bonn. 1902.
7. SOLACOLU, T., Sur les fruits parthénocarpiques. Compt. Rend. Acad. Sci. Paris **141**: 1905.
8. TREUB, M., L'action des tubes polliniques sur le développement des ovules chez les Orchidées. Ann. Jard. Bot. Buitenzorg. **3**: 1883.

DEVELOPMENT OF THE OVULATE STROBILUS AND YOUNG OVULE OF ZAMIA FLORIDANA

FRANCES GRACE SMITH

(WITH TWENTY-TWO FIGURES)

The results of a study of the development of the staminate strobilus and microsporangia of *Zamia* were published by the writer in 1907 (1). Some of the material, sent from Miami, Florida, in June of the years 1905 and 1906 for this investigation, included ovulate cones. Later in the year 1906 an effort was made to secure a complete series of young ovulate cones, for *Zamia*, alone of the cycads of North America, exists in such profusion that whole plants may be sacrificed to secure a single small cone from each. There are some stages not yet covered by the series of cones which has been obtained, but it seems worth while at this point in the study of the material to sum up the results, and to postpone conclusions from these results and their theoretical bearing upon other cycad studies until a complete series has been gathered.

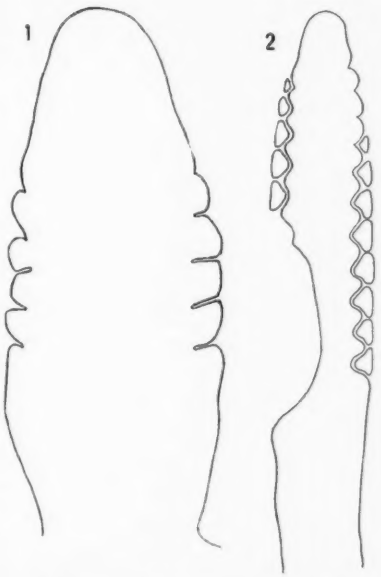
Each year, since 1906, an attempt has been made to secure material which should give the origin of the integument. In 1907, out of eight or ten plants sent from Florida between July 25 and August 8, not one contained an ovulate cone. I do not know whether this was an unfruitful year or whether the collector was unfortunate in the plants he gathered. Another year, knowing just the period during which the material ought to be gathered, careful collections were made, but in every case the cones had reached a development two weeks ahead of that of the previous year, so that it is evident collections of *Zamia* must be made often and during long periods in order to obtain a full series.

The facts ascertained from the material cover the period from the appearance of the ovulate cone to the time when the developing endosperm has partially filled the embryo sac, and will be treated under three periods of growth.

1. Development of the strobilus and the sporophylls

Plants gathered early in June show a slight elevation which elongates to form the strobilus, but at this stage it is impossible to distinguish the ovulate strobili from the staminate. As stated in my description of the staminate plants (1), the strobili are deeply sunken in the tip of the crown and are completely covered by the bases of the rosette of leaves.

A strobilus of July 5 is about 5 mm. long, and at this time can be recognized as ovulate. There are defined in a median longitudinal section five sporophylls on a side (fig. 1). A comparison with a staminate cone of July 8, with eleven sporophylls on a side, gives approximately the same length, but greater breadth and larger sporophylls than those of the staminate cone (fig. 2).



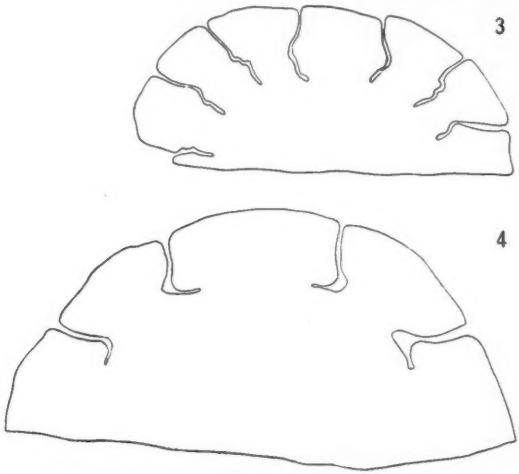
FIGS. 1, 2.—Fig. 1, longitudinal section of young ovulate strobilus, showing sporophylls (July 5). ×40; fig. 2, longitudinal section of young staminate strobilus, showing sporophylls (July 8). ×40.

In comparing cross-sections of strobili of July 25, much the same points are noticed. A staminate strobilus (fig. 3) has on an average fourteen to sixteen sporophylls on a cross-section, while the ovulate strobilus (fig. 4) has seldom more than ten. A difference in the size of the sporophylls as well as of the strobili is seen also.

The development of the sporophylls is identical in the two strobili, at least in these early stages, except that the elevation representing the sporophyll in the ovulate strobilus is broader and includes more cells in a hypodermal position which are dividing periclinally. As the sporangia appear, there are fewer meristematic points on the ovulate

sporophylls, as shown in figs. 3 and 4. The lobes themselves are broader and the cells of the inner surface show a deeper stain, indicating that this is the region where active growth is taking place. This region is included by the dotted line drawn on one of the sporophylls in fig. 4.

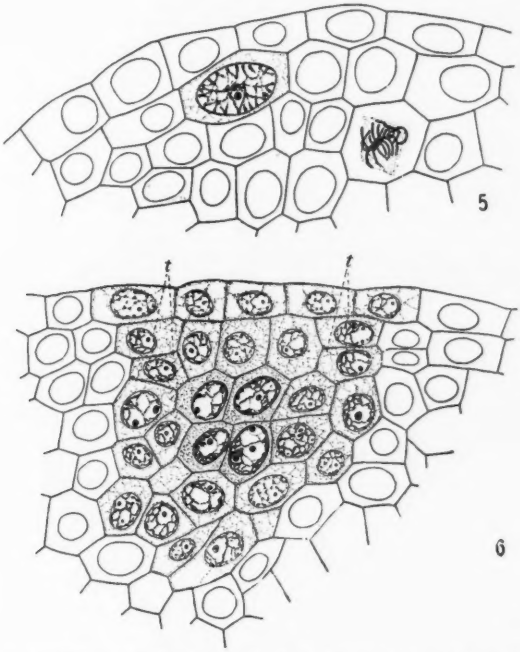
In a megasporophyll of July 25, of the size in fig. 4, a cell can be distinguished for the first time from those about it by its size and



FIGS. 3, 4.—Fig. 3, transverse section of staminate strobilus, showing sporophylls and position of sporangia (July 26). ×40; fig. 4, transverse section of ovulate strobilus (July 26), showing sporophylls and meristematic group of cells on one lobe indicated by dotted line. ×40.

by its larger nucleus with deeply staining chromatin. This cell is hypodermal in origin (fig. 5) and resembles the single archesporial cell in the staminate sporangium. About ten or twelve cells in a cross-section form a meristematic group, some of which are actively dividing, but this archesporial cell is easily distinguished from these. In another sporophyll of July 25, but evidently one a little more developed as shown by its increased size, the development of this group of cells has gone on still farther, and now four cells in cross-section can be clearly distinguished from the others (fig. 6). This group is separated from the epidermis by one layer of cells, and the

size of its nuclei and the position of its walls suggest an origin from a single archesporial cell. It resembles, too, the group in the staminate strobilus which gives rise to the sporogenous mass in the microsporangium. Two pairs of cells (*t*) just under the epidermis, from the



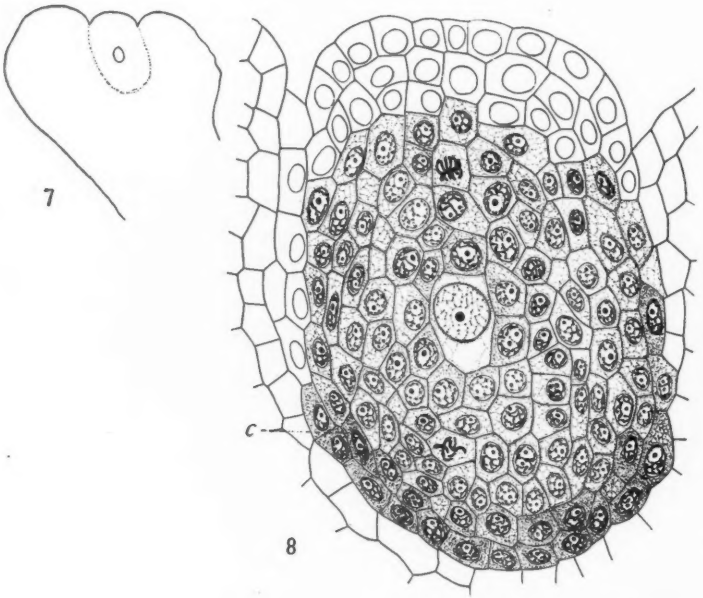
FIGS. 5, 6.—Fig. 5, detail of sporophyll of fig. 4, showing hypodermal archesporial cell. ×40; fig. 6, more advanced stage, showing group of meristematic cells (*t*) which may give rise to integument. ×1400.

direction of their walls indicate that they have arisen from periclinal divisions of hypodermal cells, and suggest the first divisions causing the elevation of the integument.

Just at this point there is the break in the continuity of the series referred to, and the strobili of August 8, which is the date of the next collection, show on either lobe of the sporophyll the projecting nucellus and the integument, which is only slightly elevated at this time.

2. Development of the ovule

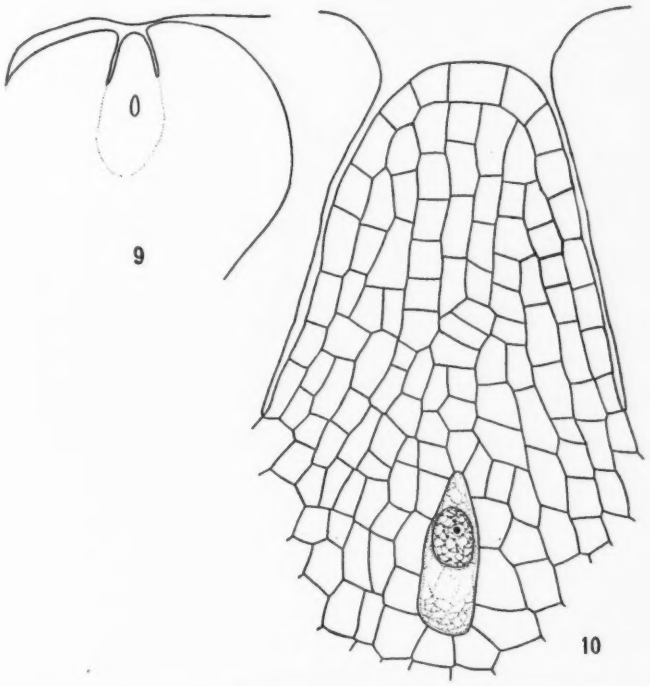
The ovule of August 8 shown in figs. 7 and 8 is younger, I think, than that of *Stangeria* as figured by LANG (**2**), and about the same age as that of *Ceratozamia* shown by TREUB (**3**). The megaspore mother cell is easily picked out at this stage as a larger cell, which is



FIGS. 7, 8.—Fig. 7, young ovule (August 8), showing (by dotted line) group of cells arising from archesporium. ×136; fig. 8, detail of fig. 7, showing megaspore mother cell, adjacent tissue dividing, and flattened cells (*c*) bounding nucellus. ×930.

more vacuolated and which has a large nucleus with the chromatin arranged in a fine network. The cells about it stain rather more deeply, and toward the chalazal end there are several rows of flattened cells (*c*) which stain still more deeply, and which form a boundary to the ovule in this direction. These rows curve up about the "sporogenous group" and meet the epidermis at the point where the integument and nucellus are separated from each other.

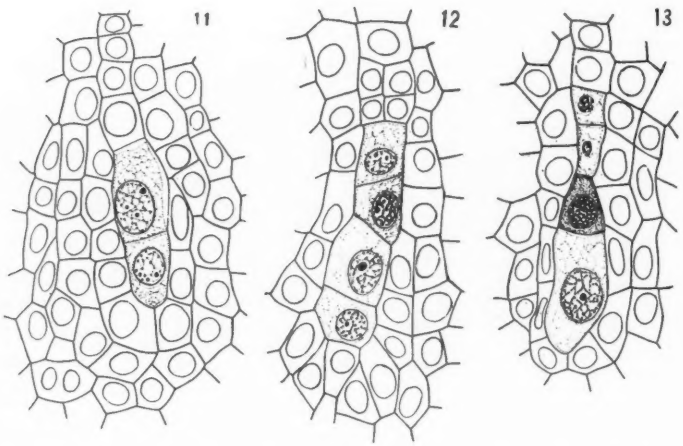
In the region of the nucellar apex, the cells are constantly dividing as the nucellus increases, but this cell activity is not confined to that part of the ovule. On either side and below the mother cell there is constant division, as is shown in sections through the mother cell



FIGS. 9, 10.—Fig. 9, older ovule, showing megaspore mother cell. ×136; fig. 10, detail of fig. 9, showing large increase in nucellar apex. ×930.

and in sections adjacent to it. Often there are three spindles in a section 8 μ thick. This megaspore mother cell enlarges somewhat before dividing again, while the nucellus by repeated periclinal divisions (figs. 9, 10) is becoming extended. This changes the broad, flat outline of the projecting portion to a narrower, more pointed one. The integument keeps pace in its development and narrows

the micropyle by growing in the direction of the nucellus. During this change in the nucellus the mother cell divides (fig. 11) into an upper larger and a lower smaller cell. The next stage seen (fig. 12) had a row of four megaspores, and the position of the upper two and again of the lower two would indicate that they were derived from these first two cells by a further division. Fig. 13 shows a disappearance of the two upper cells, indicated by the small nuclei and the

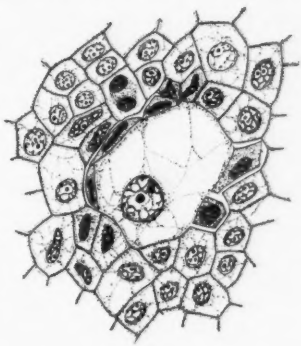


FIGS. 11, 12, 13.—Fig. 11, two cells arising from megaspore mother cell; fig. 12, chain of four megaspores. ×930; fig. 13, four megaspores: three degenerating, the fourth the embryo sac. ×930.

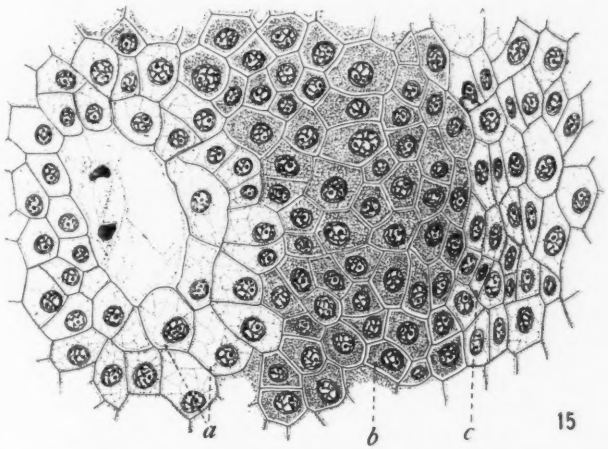
narrowed effect of the cells themselves. The nucleus of the cell just over the basal one of the chain is being flattened and crushed by the development of its sister cell, which is to be the embryo sac. In other sections there is a deeply stained cap over the micropylar end of the embryo sac, which is probably the remnant of the other megaspore or megaspores; at last this also disappears. From this time on the embryo sac enlarges rapidly and becomes more vacuolated (figs. 10, 14).

3. Development of the female gametophyte and changes in the "spongy tissue"

Many slides show the uninucleate condition of the embryo sac, indicating that this stage is quite prolonged. Fig. 14 shows a few cells surrounding the embryo sac, which are broken down by the growing sac; material killed August 29 is about the first to show this condition. The nucleus of the embryo sac divides as is described for other species, and the nuclei take the polar position. There is a slight indication of cytoplasm forming a lining to the wall, but the karyokinetic figure in the section drawn must have formed a little to the



14



15

FIGS. 14, 15.—Fig. 14, detail of enlarging embryo sac. ×930; fig. 15, detail of embryo sac and "spongy tissue" (August 29): *a*, active nutritive cells; *b*, tissue of closely packed cells; *c*, flattened cells bounding group. ×930.

side of the middle of the sac (fig. 15). Fig. 15 shows the sac and the surrounding cells on one side as far as the flattened rows (*c*)

forming the boundary of the group of cells which we may call the "spongy tissue," using STRASBURGER'S term. These flattened cells of the periphery were first seen in the young ovule (fig. 8). The "spongy tissue" may perhaps be referred to the mass which originated in a single archesporial cell, but the lack of sufficient material prevents me from drawing conclusions.

By the time the embryo sac nucleus has divided, the "spongy tissue" shows differentiation. Next to the embryo sac membrane the cells (*a*) are larger, more vacuolated, and often bulge into the sac as pressure is released on this side. Occasionally these cells are broken apart and the walls are very indistinct. The next rows (*b*), often eight or ten in number, are made up of smaller cells, therefore seeming to be closely packed and often dividing in such planes that they form rows radiating from the center of the sac. The nuclei and cells themselves stain deeply, contrasting both with the inner layers mentioned and with the flattened peripheral rows (*c*). Quite often a spindle is seen in these cells, so that this is by no means a degenerating tissue. The cytoplasm in the preparations has separated from the walls a little, and the walls are "so transparent that the nuclei seem to be floating in cytoplasm" (3), but I am convinced that this is due to a slow passage of the killing fluid into the tissue and the consequent shrinkage. Fig. 16 shows the two embryo sac nuclei at the poles, and fig. 17 has four nuclei in one section, one of which has not yet passed to the peripheral position. The interior of the sac in these sections has a beautiful vacuolate structure, and the "spongy tissue" has not changed appreciably.

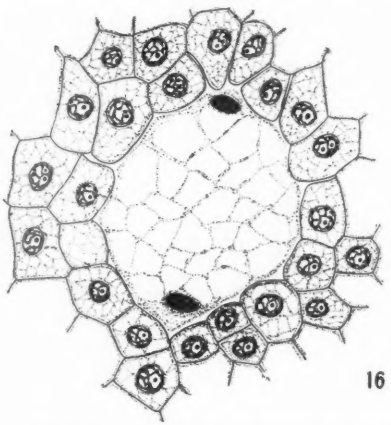
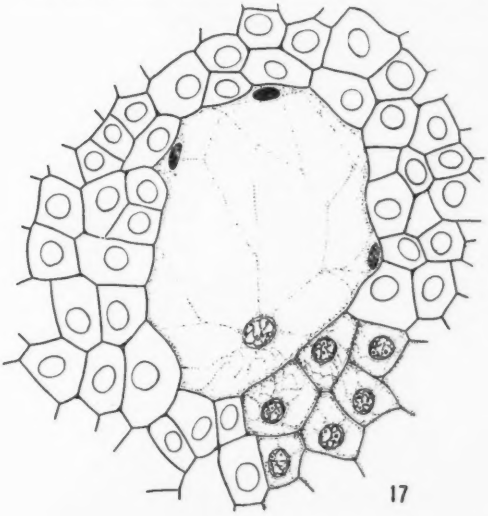
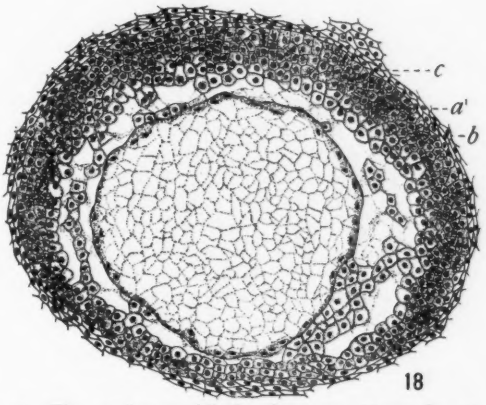


FIG. 16.—Polar position of embryo sac nuclei. ×930.

The next series of slides obtained give further divisions of free nuclei and their peripheral position in the cytoplasmic lining, which



17

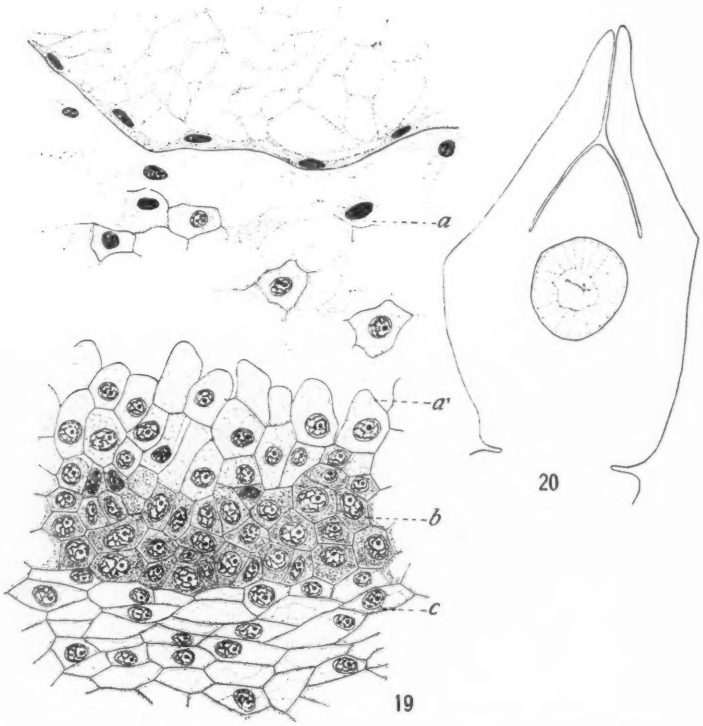


18

FIGS. 17, 18.—Fig. 17, four nuclei of embryo sac. ×930; fig. 18, embryo sac with layer of cells; spongy tissue beginning to break down. ×250.

is now a little thicker. The cytoplasm here is quite foamlike in its structure (figs. 18, 19). Fig. 19 is a detail of the sac and the "spongy

tissue" at the micropylar end. The result of the growth of the sac is seen in the breaking down of the "spongy tissue"; the layers nearest the sac are almost used up (*a*). Seemingly the wall of each cell is



FIGS. 19, 20.—Fig. 19, detail of fig. 18: *a*, "spongy tissue" broken down; *a'*, nutritive tissue; *b*, compact tissue; *c*, flattened cells. ×930; fig. 20, ovule showing unusual development of "spongy tissue." ×37.

attacked first, probably by an enzyme secreted by the developing gametophyte, and the cells become separated from each other. Sometimes a nucleus has its position in an isolated cell, and sometimes it is surrounded by cytoplasm. The breaking down of this tissue resembles that described by CHAMBERLAIN (**4**) in the formation of the pollen chamber of *Dioon edule*.

Next to this broken tissue there are several layers of cells (*a'*) which resemble those in fig. 16 immediately surrounding the sac. These are swollen and have every appearance of being active, nutritive cells. Beyond this again is the deeply staining tissue (*b*), but narrower now, as if some of the cells had changed in their appearance and had taken

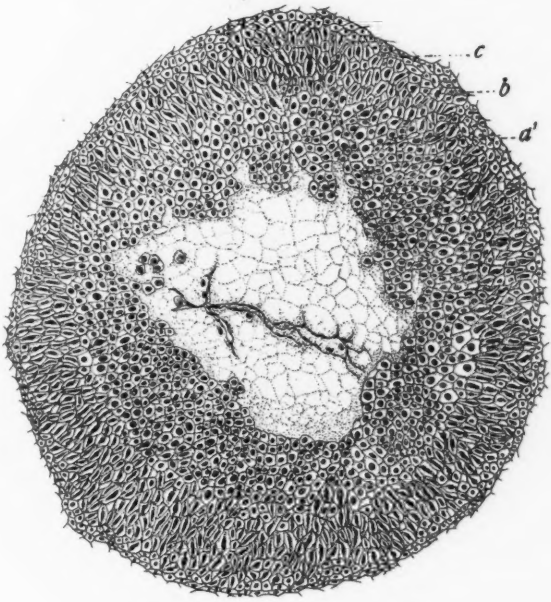


FIG. 21.—Detail of fig. 20. ×250.

on the function of the cells which previously bordered the sac. This tissue is narrower still toward the chalazal end of the sac.

Figs. 20 and 21 were made from an ovule in which the embryo sac was shrunken badly, but the amount of "spongy tissue" formed was unusually large and compact. The outer part shows radiation in its cell rows, as in other earlier stages. In one case this increase of "spongy tissue" was so great that it almost filled the interior, and the embryo sac had not developed. All these facts point to an active "spongy tissue" such as Miss FERGUSON claims for *Pinus* (**5**), and not

one which is formed early and which disappears early, leaving only a few thin layers, called "tapetum" by LANG (**3**) and not explained.

In fig. 22 this encroachment upon the "spongy tissue" has gone on until only about three or four rows are left between the sac and the bounding narrow cells (*c*). These inner cells have enlarged many times, as is shown by the magnification of the figures recorded. The walls of these cells (*b*) have become quite thick, the cells are full

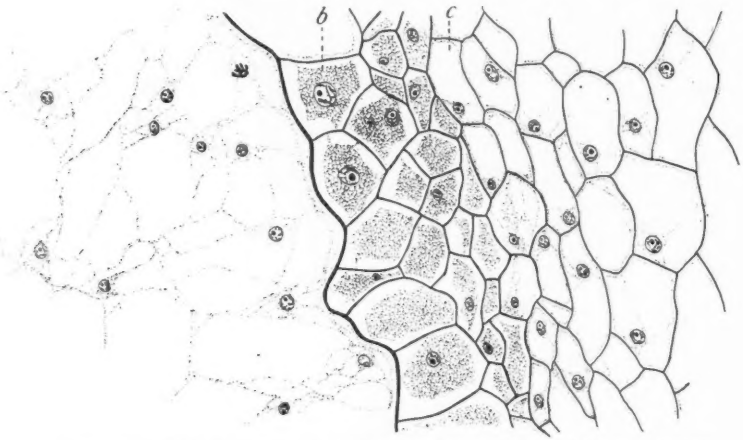


FIG. 22.—Detail of embryo sac with many free nuclei; diminished "spongy tissue"; cells of tissue (*b*) have increased in size and assumed nutritive function; *c*, flattened cells. ×580.

of cytoplasm, and the nuclei are not large, though not disorganized. The sac has now a broad cytoplasmic layer and a heavy wall. The nuclei are scattered everywhere, but have no walls as yet; at least none were seen in a careful examination with a $\frac{1}{12}$ oil immersion lens. The membrane is drawn away from the "spongy tissue" for a little distance at the micropylar end, so that in cutting the ovule it looks as if this end of the sac was not filled in.

From this stage on, the history of the development of the sac has been fully worked out in other forms by WARMING (**6**), whose description of the endosperm formation is excellent, by IKENO (**7**), and by others, so that it did not seem worth while to examine later material, at least not until the gaps behind could be closed up.

Summary

1. The young staminate and ovulate strobili can be distinguished by the difference in breadth, number of sporophylls, and number of meristematic points.

2. There is probably a single archesporial cell giving rise to a group of cells, one of which becomes the megaspore mother cell.

3. There are four potential megaspores, the lowest one becoming the embryo sac, whose development agrees with the accounts of other cycads in the main points.

4. The "spongy tissue" is an active, nutritive tissue, adding to its width by division of its cells as it is encroached upon by the embryo sac.

5. In its final degeneration, the cells of the "spongy tissue" nearest the embryo sac are first attacked, and the smaller cells outside them take their place, becoming large, swollen, and nutritive in function.

SMITH COLLEGE
NORTHAMPTON, MASS.

LITERATURE CITED

1. SMITH, FRANCES GRACE, Morphology of the trunk and development of the microsporangium of cycads. BOT. GAZETTE **43**:187–204. *pl. 10.* 1907.
2. LANG, W. H., Studies in the development and morphology of cycadean sporangia. II. The ovule of *Stangeria paradoxa*. Annals of Botany **14**: 281–306. *pls. 17, 18.* 1900.
3. TREUB, M., Recherches sur les Cycadées. III. Embryogénie du *Cycas circinalis*. Ann. Jard. Buitenzorg **4**:1–11. *pls. 1–3.* 1884.
4. CHAMBERLAIN, C. J., The ovule and female gametophyte of *Dioon edule*. BOT. GAZETTE **42**:321–356. *pls. 13–15.* 1906.
5. FERGUSON, MARGARET, The spongy tissue of STRASBURGER. Science N.S. **18**:308–311. 1903.
6. WARMING, E., Undersogelser og Betragtninger over Cycadeerne. Oversight K. Vidensk. Selsk. Forh. pp. 100, 101. 1877.
7. IKENO, S., Untersuchungen über die Entwickelung der Geschlechtorgane und der Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. Wiss. Bot. **32**: 557–602. *pls. 8–10.* 1889.

OXIDIZING ENZYMES AND THEIR RELATION TO "SAP STAIN" IN LUMBER[1]

IRVING W. BAILEY

The discoloration of sap wood, or "sap stain" as it is commonly called, involves the loss or the depreciation in value of large quantities of lumber annually. Therefore, with the continually increasing value of lumber and the necessity for greater economy in the utilization of wood, the problem of saving and using the sap wood as well as the heart wood of the tree increases in importance each year. In endeavoring to prevent the discoloration of sap wood it is of fundamental importance to discover what agency or agencies produce the stain, and to study their mode of activity.

So far as the writer has been able to determine, there are in general two agencies which produce the discoloration of sap wood. One of these, which is of frequent occurrence and of great economic importance, is a purely chemical reaction which takes place in green sap lumber upon exposure to the air (oxygen). This chemical discoloration occurs in many varieties of wood, and is well illustrated by the reddish yellow or rusty colored sap stain which occurs in the sap wood of alder, birch, and cherry, and by the blue colored sap stain which occurs in the sap wood of red gum (*Liquidambar*). When the freshly cut surfaces of these woods are exposed to the air, under favorable conditions of temperature and moisture, a chemical reaction starts which with varying rapidity produces a colored substance in the wood. Favorable conditions for sap-staining are found during warm weather, and optimum conditions during extremely hot, humid, summer weather, when lumber becomes discolored within a few hours. The examination of microscopic sections of this sap-stained lumber reveals the fact that the colored substance, produced by the chemical reaction, is most conspicuously developed in the wood rays and wood parenchyma cells, living tissues which are largely concerned in the storage and conduction of food in the wood. The

[1] Contributions from the Phanerogamic Laboratories of Harvard University. No. 26.

second agency which produces discoloration in sap wood is the activity of fungi, which find an abundant food supply in the sap wood, and under favorable conditions of heat and moisture (hot, humid weather) develop with great rapidity. Thus part at least of the blue colored sap stain which occurs so frequently in pine may be produced by the dark colored mycelium of a fungus which is found in the food-containing wood rays and the parenchyma cells surrounding the resin canals. In fact, in all samples of blue sap pine which have come under my observation the color has been produced by this dark colored mycelium. However, it seems very likely that a large part of the blue sap stain in pine is produced by chemical reactions, for I am informed by lumbermen that sap pine lumber frequently stains badly during a few hours. The rapidity with which the discoloration is produced indicates the activity of a chemical reaction.

Although the two agencies producing sap stain are so fundamentally different, yet the conditions which favor their activity are very similar. As has been indicated above, each agency, in its activity, is closely related to the food substances contained in the wood. This is shown by the fact that the discolorations, produced by the activity of fungi and by chemical reaction, are most conspicuously developed in the wood rays and wood parenchyma cells. Both agencies producing sap stain are, in addition, dependent upon certain quantities of oxygen (air), heat, and moisture. Thus optimum conditions for sap-staining are found in green sap lumber during hot, humid weather, whereas unfavorable conditions are found in cold, dry weather and in logs immersed in water. It is well known that certain quantities of food, oxygen, moisture, and high temperature are necessary for the rapid development of fungi, but it seems advantageous to examine with greater care a chemical reaction which is dependent upon similar conditions.

Oxidizing enzymes

It has been known for many years that certain soluble ferments, which facilitate the oxidation of organic compounds, are widely distributed in plants and animals. These oxidizing enzymes are of fundamental importance in the nutrition and growth of living organisms. Furthermore, it has been clearly demonstrated that these enzymes

produce certain striking postmortem changes in animal and plant tissues. I refer in particular to the postmortem discolorations of certain organic compounds which are produced by the oxidizing activity of these enzymes. The most striking illustration of this oxidation and discoloration in plants was pointed out, in 1883, by a Japanese, HIKOROKURO YASHIDA, who discovered that the formation of black varnish by the oxidation of the latex of the lac tree was produced by the intervention of an oxidizing ferment. Since this discovery, the activity of oxidizing enzymes has been carefully studied in the discolorations which are produced by them in the extracted juices of fruits, vegetables, cereals, mushrooms, and other soft plant tissues. These investigations have shown conclusively that, as in the case of the oxidation of the latex of the lac tree, postmortem discolorations are produced in these juices by the intervention of oxidizing ferments. This change of color which is produced in the extracts of plant tissues has been used as the basis for very delicate tests for oxidizing ferments. A strong blue color produced in an alcoholic tincture of guaiacum, in the presence of oxygen or hydrogen peroxid, indicates the presence of oxidizing enzymes. Upon the basis of these tests certain authorities divide oxidizing ferments into oxidases and peroxidases, according to whether the blue color produced in the tincture of guaiacum occurs in the presence of oxygen or hydrogen peroxid.

It is of interest to note the behavior of oxidizing ferments under variations of temperature. Investigation has shown that optimum temperatures exist for oxidizing enzymes at which they react with great activity. Below these optimum temperatures their activity decreases, and similarly in increasing the temperature above the optimum their activity decreases, and in almost every case their activity is entirely destroyed before a temperature of 100 C. is attained. The activity of oxidizing ferments is also decreased or destroyed by certain antiseptics, and by other chemical substances.

From this consideration of certain properties of oxidizing enzymes, we see that there exists a strong similarity between their oxidizing activity and the chemical reactions which produce sap stain. In each case postmortem oxidization with change of color is produced by solutions in contact with the air (oxygen), and variations in tempera-

ture produce similar variations in the activity of the discoloring agency.

Prevention of sap stain

The fact that the temperature of boiling water destroys the activity of oxidases and peroxidases suggested to the writer the idea, that if sap stain were produced through the action of oxidizing enzymes, a simple method of preventing the discoloration of sap wood might be secured by immersing lumber in boiling water. During the spring of the present year the writer has tested this method on small boards (1″×3″×6″) of *Alnus incana* Moench., *Betula populifolia* Marsh., *B. papyrifera* Marsh., and various rosaceous trees. These species were selected as, at this latitude (Massachusetts), the sap wood stains very easily and rapidly during the spring upon exposure to the air, whereas the sap wood of such trees as sweet gum, maple, and basswood stain deeply during extremely hot and humid summer weather. The result of these tests has been to show that boards immersed in boiling water for a few minutes remain permanently unchanged in color, whereas untreated pieces stain rapidly and deeply. The fact that the unboiled boards stained on exposure to the air and that the discoloration was prevented by boiling offered strong evidence for believing that sap stain results from the action of oxidizing ferments. On testing boiled and unboiled boards with the guaiacum tests, the blue color produced in the unboiled boards demonstrated the presence of oxidizing enzymes.

In conducting these experiments the boards which were to be boiled and those which were to serve as controls were in all cases cut from the same part of the same tree, and were subsequently exposed to the same atmospheric conditions of temperature and humidity. The extreme sensitiveness of oxidizing enzymes in their oxidizing activity was clearly demonstrated by the species of wood used in these tests. For example, alder and birch, which in hot, humid weather stain in a few minutes to a reddish yellow or rusty color, stain more slowly at lower temperatures, and in cold dry weather stain but slightly even after an interval of several weeks. In a similar way the depth to which the stain penetrates into the wood varies with temperature and moisture. Boards dried in sheds stain superficially,

whereas boards dried in damp conditions in the open stain very deeply and densely. Boards treated in hot water and dried under cover remain unchanged in color throughout, and those placed in the most unfavorable conditions of abundant moisture and high temperature in the open, although they scorch superficially, as does all lumber exposed to the direct rays of the sun, remain unstained beneath this thin layer of tan.

Practicability of hot water treatment

It will occur to some readers that the activity of oxidizing enzymes could be prevented by kiln drying or steaming the wood, and that one of these methods would be more practicable than immersion in boiling water. However, to those familiar with saw mill practice it is evident that in mills sawing many thousand board feet of lumber each day and night it is usually difficult to steam or kiln dry more than a small percentage of the cut. Steaming or kiln drying are expensive processes, and the latter can only be applied to certain varieties and grades of wood. Furthermore, these processes are time consuming and require much handling of the lumber. Some method must be devised for preventing sap stain which can be carried out very cheaply and rapidly, in order not to interfere with the movement of the lumber from the saw to the yard. In other words, the lumber must be removed, treated, and piled as rapidly as it is sawed, in order not to interfere with the daily output of the mill. Some of the larger mills in the south have installed tanks containing chemical substances which destroy the action of oxidases and prevent discoloration by fungi. The lumber as it comes from the saw is carried through this tank upon carrier chains and thus immersed and coated with the chemicals. Certain difficulties have been encountered in the use of this method. One of the most serious is the fact that the chemicals used fire proof the outer surface of the boards, and when the treated lumber is shipped to northern markets for finishing the planings and shavings cannot be burned. This obliges the use of coal to run the planers and other machinery, and the non-combustible planings must be disposed of.

In the treatment of lumber with boiling water a similar method could be employed. Long shallow tanks in which water is heated

by steam pipes or by steam exhausts could be arranged so that the lumber from the saw could be passed through the tanks on carrier chains.

During the coming summer the writer will extend his experiments to include a wider variety of woods, and will conduct experiments of a practical nature in a large southern saw mill, to determine the relative advantages of hot water treatment and treatment with chemical substances.

Summary and conclusions

1. Sap stain is in general produced in two ways, by the attacks of fungi and by chemical discoloration.

2. Chemical discoloration is produced in sap wood by the activity of oxidizing enzymes.

3. Hot, humid weather is very favorable to the activity of these ferments, and cold winter weather is unfavorable.

4. Oxidizing enzymes which produce sap stain in wood are destroyed and their oxidizing action prevented by a temperature of 100° C.

5. Treating the sap wood of alder, birch, and cherry with boiling water destroys the oxidizing enzymes in the wood and prevents sap stain.

6. Treating sap lumber in long tanks of boiling water appears to be a practical method of preventing sap stain, and to be well adapted to saw mill practice.

In conclusion the writer wishes to express his sincere thanks to Professor E. C. JEFFREY for valuable assistance in carrying out this investigation, to Professor G. E. OSTERHOUT for advice and suggestions, and to MR. W. R. BUTLER for material of sap-stained lumber.

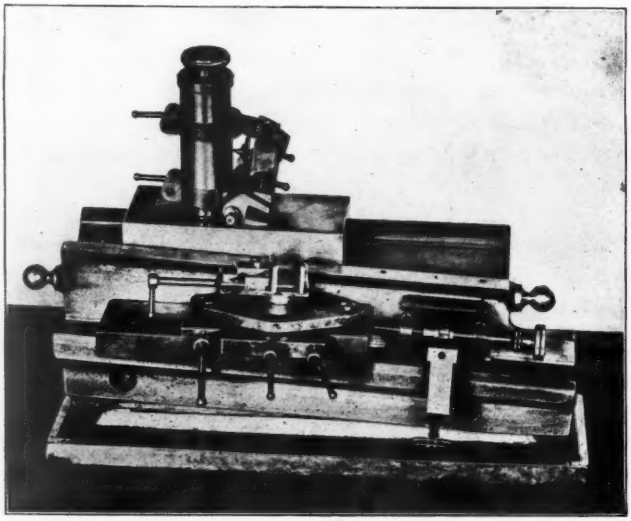
HARVARD UNIVERSITY

BRIEFER ARTICLES

A MODIFICATION OF A JUNG–THOMA SLIDING MICROTOME FOR CUTTING WOOD

(WITH ONE FIGURE)

The object and knife carrying blocks are long and heavy. These have planed surfaces of contact with the running surfaces of the base, instead of being mounted on ivory bearings, as is the case in the Thoma instrument. When oiled, the blocks are held by capillarity so firmly to the tracks that



the whole instrument can be suspended by either block without breaking the contact. In this way rigidity is secured and vibration eliminated, so far as the running surfaces are concerned. The friction of running is less than in the ordinary Thoma.

The object clamp is mounted on a solid ball (1.25 inches diameter), in a tight socket. This ball can be revolved horizontally through 180° and vertically through about 45°. It has two orienting levers whose axes

of contact with the ball are at right angles to each other, so that *definite orientation* of the object in either of *two* planes is possible. A third lever draws the plate which fits around the "shoulder" of the ball down when correct orientation has been attained, and makes the whole absolutely rigid.

The knife carrier revolves horizontally on the column of the knife block, and is rapidly adjustable to a height of 1.25 inches by means of the screw at the top, the two levers behind clamping it instantly in place. Arrangement has also been made for the adjustment of the vertical angle of the knife.

The rigidity and rapidity of the adjustment of this instrument are found invaluable in cutting many and long series of wood sections. Its usefulness was demonstrated in the sectioning for Mr. W. P. THOMPSON'S work on the rays of the conifers recorded in this issue of the BOTANICAL GAZETTE, and the structure of the instrument is outlined here in the hope that others may find it useful.

No small amount of the credit for the performance of the instrument is due to the excellent workmanship of Mr. H. W. SPENCE, who also assisted in the designing.—R. B. THOMSON, *University of Toronto.*

CURRENT LITERATURE

BOOK REVIEWS

The oxidases

KASTLE[1] has recently published a monograph on oxidases and other oxygen catalysts concerned in biological oxidations. The work is a compilation of our present knowledge concerning oxidases and other oxygen catalysts, as well as an excellent historical résumé of the subject. The constantly growing recognition of the important rôle of oxidases and related oxygen catalysts in biological processes, as well as the rapidly growing literature on the subject, makes a summary of the real contributions especially valuable at the present time.

The first chapter deals with the important past and present theories of oxidation, beginning with SCHOENBEIN'S ozone theory. After a brief discussion of this theory, the author proceeds with a more detailed account of VANT' HOFF'S theory of ionization, HOPPE SEYLER'S nascent hydrogen theory, and the peroxid theory of TRAUBE, ENGLER, and BACH. The conception which involves an exchange of electrical potential in oxidations, however, is not mentioned.

The second chapter takes up the oxidizing ferments, and begins with a discussion of their rôle and range in biochemical processes. This is followed by a detailed account of the guaicum reaction, since our first knowledge of oxidizing ferments is so closely associated with the reaction. The historical treatment of oxygen exciters and oxygen carriers is divided into two periods. The first covers the first sixty years of the nineteenth century, with SCHOENBEIN'S contributions standing out as the most important. He supposed that by means of various substances and under various influences the oxygen of the air becomes ozonized. These substances may in turn combine with ozone thus produced to form an active ozonid, which in turn can give up its oxygen to other less readily oxidizable substances. Thus the presence of oxygen activators and carriers was recognized, and the most important characteristics of oxidases and peroxidases discovered, although up to this time these terms had not been introduced into science.

During the second historical period, the contributions of TRAUBE and BERTRAND are especially emphasized. TRAUBE in his *Theorie der Fermentwirkungen* (1858) established the chemical entity of oxidizing ferments and their importance in acting as chemical go-betweens between free or combined oxygen

[1] KASTLE, J. H., The oxidases and other oxygen catalysts concerned in biological oxidations. U.S. Public Health and Marine Hospital Service, Hygienic Laboratory, Bull. 59. 1909.

and the fermentable substances. To BERTRAND we owe the introduction into science of the term oxidase. The adoption of this general term was based upon the discovery and characteristics of laccase and tyrosinase. The chapter concludes with a classification of oxidases and special reference to the sources, preparation, and characteristics of laccase, tyrosinase, aldehydase, and the purin oxidases.

The third chapter is devoted to the peroxidases and catalases. The weight of opinion is inclined to the conception that peroxidases are substances capable of forming unstable peroxids from hydrogen peroxid, by double decomposition or by combining directly with the hydrogen peroxid to form unstable holoxid (TRAUBE) derivatives, possessing greater powers of oxidation than hydrogen peroxid.

BACH and CHODAT's conception of an oxidase consisting of a mixture of peroxidases and peroxid-forming substances (oxygenases) would make the peroxidases the more important agents in plant and animal oxidations, and would relegate the oxidases to an insignificant position in such oxidations, if indeed they function as enzymes at all. In the author's opinion the objections which have been recently urged against the true enzymatic nature of oxidases are well taken.

A considerable amount of evidence is brought together to show the importance of iron, copper, and manganese as coenzymes to oxidizing ferments. According to BERTRAND, manganese is the really active element of the oxidases, so far as the activation and transfer of oxygen is concerned. EULER and BOLIN have found that laccase has no action on hydroquinone in the absence of manganous salts, and therefore they suggest that laccase owes its activity to the presence of such salts. In this connection it is interesting to note that in a paper by BACH,[2] more recent than the above monograph, he claims to have obtained a tyrosinase which will oxidize tyrosin to the red stage and is free from both iron and manganese. He concludes, therefore, that manganese and iron salts are in no way necessary for oxidase activity. One of the most valuable features of the monograph is the comprehensive list of references to the literature.—CHARLES O. APPLEMAN.

Colloidal chemistry

The newer plant physiology should welcome the appearance of FREUNDLICH'S book[3] on colloidal chemistry, or capillary chemistry, as he terms it. This is the first attempt to bring together our knowledge of this youngest and most difficult branch of physical chemistry. It puts the physiologist immediately in touch with the present status and most important literature of a subject which seems destined to play at least as important a rôle in the study of vital phenomena as

[2] BACH, A., Zur Theorie der Oxidasewirkung. Ber. Deutsch. Chem. Gesell. **43**:362. 1910.

[3] FREUNDLICH, HERBERT, Kapillarchemie, eine Darstellung der Chemie der Kolloide und verwandter Gebiete. 8vo. pp. viii + 591. *figs.* 75. Leipzig. 1909.

has the chemistry of true solutions. The importance of surface tension in the mechanics of cell life has been emphasized, adsorption phenomena have recently become important in our consideration of semipermeability, selective absorption, etc., they must also be called upon to explain many of the relations between soil and plant. The student of protoplasmic structures, nuclear membranes, chromosomes, fibrillae, and the rest, is perhaps more familiar with coagulation of colloids and adsorption than he knows; his killing and fixing are examples of the former, while the whole process of staining apparently depends upon the different adsorptive powers exhibited by various portions of the coagulated protoplasmic mass. The so-called Brownian movement and the other phenomena of motion usually observed in the protoplasmic emulsion are likewise to be classed under capillary chemistry. If enzyme action is to receive an explanation, it bids fair to come also from this realm.

With this book and the field that it represents once in general use, it would seem that physiological research should receive a very great impetus along just those lines where it now wavers most. One of imagination, who appreciates the problems and present rapid advance of this and other branches of physical chemistry, should have little cause so thoroughly to lose heart as to need the aid of those "entelechies" and other *dei ex machina* with which the "neo-vitalism" seems to be somewhat overburdening biological philosophy. In the present exposition of capillary chemistry the author proceeds from the simpler phenomena of surface tension and capillarity to subjects of more complex nature, such as adsorption, colloidal solutions, suspensions, emulsions, catalysis, and the like. Every section is brief, clear, and directly to the point in hand; experimental evidence is given prominence rather than theoretical deductions, though the latter are not wanting; and numerous footnotes orient the reader in the scattered literature of the subject. An index of authors and one of subjects enhances the value of the work.—B. E. LIVINGSTON.

MINOR NOTICES

Insect galls.—Miss STEBBINS[4] has published a bulletin on insect galls of Springfield, Mass., and vicinity, which will be very useful to botanists who are interested in cecidology. The galls are grouped with reference to the plants, which have been arranged in accordance with BRITTON'S *Manual*. This is the first American work in which these pathological growths have been grouped with reference to the host plants. The record shows 204 species of gall-producing insects, which are distributed in 52 genera, 14 families, and 6 orders. The galls occur on 93 species of host plants, which are distributed among 48 genera, 29 families, and 16 orders. The descriptions of the galls are clear and are reinforced by 112 illustrations. The descriptions of the insects are omitted, but the synonomy and bibliography given with each will enable the student of entomology to look them up without difficulty. The galls of 26 new species are described and named,

[4] STEBBINS, FANNIE A., Springfield Museum of Natural History, Bulletin 2. 1910.

and 6 are described without names. The describing and naming of new species of galls without the insects has been the subject of considerable criticism, but since it gives us a definite record of these species the reviewer is inclined to favor the violation of this law of nomenclature. The work closes with an extensive bibliography, a systematic index of gall insects, and an index of scientific and common names of host plants.—MEL T. COOK.

NOTES FOR STUDENTS

Influence of environment on wheat.—One of the most persistent theories in evolutionary discussions of cultivated plants is that of the "breaking up of types," supposed to be brought about when plants are grown from seed under conditions differing markedly from those under which the parent plants were grown. Evidence for this view has been largely of an observational nature and capable of other interpretation. Experimental evidence bearing on the question has been brought out by LECLERC and LEAVITT[5] in reporting a series of cultures of wheat in widely different sections of the United States. The plan of the experiments was as follows: In one series Kubanka wheat grown in South Dakota was distributed to stations in Kansas and California, a sample being likewise grown in South Dakota. Every year a sample from each station was sent to each of the others and grown there. A similar series of cultures was carried out with Crimean wheat in Kansas, Texas, and California. Some of the experiments have now been continued for five years.

The results may be briefly summarized. The original pure type of Kubanka wheat from South Dakota showed entirely different morphological characteristics and chemical composition at the different stations. The characteristics of the wheats of one variety at any particular station were uniform for the wheat whenever grown at that station, no matter from which station the seed had been derived. Thus, when South Dakota wheat was grown in Kansas or California, it assumed characteristics different from those which it originally had, and peculiar for each region; but if, after several generations, these wheats were again transferred to South Dakota the resulting crop assumed all the characteristics of the same variety grown continuously in South Dakota. The series with Crimean wheat gave exactly similar results.

The experiments show that wheats of one variety from several sources, when grown in the same locality, differ but little in morphological characteristics and chemical composition, but if grown in different localities from seed of the same source, they differ widely from each other. There is a marked response to environment, but all the plants of a pure variety respond in the same way. There is no tendency toward "breaking up" of the type on account of change in environment.—H. HASSELBRING.

---

[5] LE CLERC, J. A., and LEAVITT, S., Tri-local experiments on the influence of environment on the composition of wheat. U.S. Dept. Agr., Bur. Chem., Bull. 128. pp. 18. 1910.

Alcoholic fermentation.—KOHL[6] has carried on a study of the series of reactions involved in alcoholic fermentation. He finds that lactic acid is not fermented either by zymase, compressed yeast, or bottom yeast; that 1 per cent or more of lactic acid stops the self-fermentation of living yeast and strongly reduces its fermentative activity in glucose; but that zymase, compressed yeast, and brewer's yeast ferment sodium lactate speedily. It is evident that if at one stage of alcoholic fermentation lactic acid is found, it must exist as a salt. The fact that zymase will not ferment lactic acid has been urged against BUCHNER's conception that alcoholic fermentation takes place in two steps; glucose is transformed to lactic acid by zymase, and lactic acid to alcohol and carbon dioxid by lacticidase. KOHL's finding answers this argument. While he thinks the fermentation occurs in these two steps, he differs in his view of the enzymes that carry on the processes. He concludes that catalase transforms the glucose to lactic acid, and that zymase carries the splitting on to alcohol and carbon dioxid. In a glycerin extract of crushed yeast, he found neither an oxidase nor a peroxidase, as shown by an alcoholic solution of quaiac; yet it contained an enzyme capable of oxidizing various phenols, and these oxidations he believes are carried on by the catalase present. This extract when filtered produces lactic acid in the presence of glucose—it likewise produces a trace of oxalic acid. He does not know whether both these oxidations are due to the same enzyme. He urges that this view locates the function of the catalase of yeast, a point not before settled. In case zymase is present, the oxidations go no farther than lactic acid, which is then transformed to alcohol and carbon dioxid. In its absence, the oxidation is carried still farther, producing various other acids. He applies his view to the explanation of the results of HARDEN and YOUNG with the gelatin filter, but cannot be said in any degree to further elucidate them. This subject, because of its close bearing on respiration and energy-production in the organism, certainly needs much attention from biological chemists. It is disappointing, however, that the contributions are mainly hypotheses with sparse experimental evidence, rather than records of careful chemical studies.—WILLIAM CROCKER.

Adoxa moschatellina.—The uncertain systematic position of *Adoxa* induced LAGERBERG[7] to undertake a complete morphological and cytological investigation of this peculiar genus. The development of the various organs was traced, and this comparatively simple part of the study is illustrated by figures in the text; while the cytological details of spermatogenesis, oogenesis, and fertilization are illustrated by three large plates. The following are some of the principal features: The ovule has a single integument and a single archesporial cell which

[6] KOHL, F. G., Ueber das Wesen der Alkoholgärung. Beih. Bot. Centralbl. **29**[1]:115–126. 1910.

[7] LAGERBERG, T., Studien über die Entwickelungsgeschichte und systematische Stellung von *Adoxa moschatellina*. Kungl. Sv. Vet. Akad. Handl. **44**:1–86. *pls. 1–3. figs. 23.* 1909.

develops directly into the embryo sac, as in *Lilium*, the four megaspores, not separated by walls, all taking part in the formation of the sac. The two male cells retain their form even after passing to the end of the pollen tube. Double fertilization was observed. The diploid and haploid chromosome numbers are 38 and 18. The first four cells of the endosperm are long and tubelike, extending from the egg to the antipodals.

Various genera of the Saxifragaceae, Araliaceae, Caprifoliaceae, and Ranunculaceae, with which various systematists have supposed *Adoxa* to be related, were studied for comparison, and one of these genera (*Sambucus*) shows so many resemblances that the similarity could hardly be accidental. For instance, both have ovules with a single integument and a single archesporial cell which develops into the embryo sac according to the *Lilium* type; the wall of the anther, the cytological details of the development of the pollen (including the number of chromosomes), and the structure of the mature pollen grain are so identical that the two forms can hardly be distinguished in these respects; the long persistence of the organized male cells is the same in both, and resemblances in the grosser morphology were already well known. The conclusion is reached, and it seems to be based upon an unusually wide range of evidence, that there is no need for the family Adoxaceae, and that *Adoxa* should be placed in the Caprifoliaceae in the tribe Sambuceae.—CHARLES J. CHAMBERLAIN.

Geotropism.—Under a very pretentious title, GILTAY[8] discusses and describes a number of experiments on some of the "fundamental questions of geotropism." The article is more a contribution to the teaching of the subject than to knowledge. He gives an excellent method for lecture demonstration of the force with which a geotropically bending primary root turns downward. He also points out that we have never proved that gravity is the only stimulus involved in the turning of the primary root toward the center of the earth. The only evidence we have for this is qualitative. KNIGHT showed (1806) that as the centrifugal force was increased on a centrifuge with a vertical axis the root and stem took more nearly the horizontal position; but he did not show any relation between the position of the stem and root and the resultant of the two forces which he assumed to be involved (gravity and centrifugal force). In short, KNIGHT showed that at least in part the so-called geotropic stimulus is the gravity stimulus, but he did not show that the gravity stimulus is the only stimulus involved. GILTAY urges the necessity of showing that the position taken by orthotropic organs on such a centrifuge is the resultant of the two forces, if we are to be assured that the geotropic stimulus is identical in nature with the gravity stimulus and with the stimulus of centrifugal force. The resultant of the two forces ought of course to be the position taken, provided the two forces do not differ from each other by many fold, for we must remember that WEBER'S

[8] GILTAY, E., Einige Betrachtungen und Versuche über Grundfragen beim Geotropismus der Wurzel. Zeitschr. Botanik **2**:305–331. 1910.

law applies in geotropism as in various tropic responses. GILTAY devised a special centrifuge with a vertical axis for testing this point. He finds that with the average of 368 tests, with the angle of the resultant approximating 45°, the primary root fell 2.1° below the resultant. This deviation can well be accounted for by the variation in speed of rotation and the variation of roots themselves. This seems to furnish evidence for the identity in nature of the geotropic and centrifugal stimuli.—WILLIAM CROCKER.

Rôle of hydrocyanic acid.—TREUB[9] has found that the amount of hydrocyanic acid in plants of *Sorghum* increases during the day, not due to the direct action of light, but in proportion to the formation of the products of the assimilation of carbon. It was already known, from investigations with *Pangium edule* and *Phaseolus lunatus*, that light has no part in the formation of hydrocyanic acid except as it favors photosynthesis. Much the same results have been obtained with *Prunus javanica*. *Passiflora foetida* and at least four other plants offer examples for the demonstration of the direct proportion between the formation of hydrocyanic acid and the function of chlorophyll. This can be demonstrated also by the use of variegated leaves. The amount of acid is usually greatest in the young leaves and gradually diminishes as the leaves grow older. With *Sorghum*, young leaves grown in a dry season or on dry soil contain much acid, and for this reason are dangerous as food for stock. Leaves about to fall contain very little acid, while, with only two exceptions, those already fallen contain none. GUIGNARD found that fallen leaves of *Sambucus nigra* contain much of the acid. TREUB confirms these results and finds the same to be true of fallen leaves of *Indigofera galegoides*. The hydrocyanic acid is probably the first recognizable simple organic product of the assimilation of nitrogen, and perhaps the first organic nitrogen compound formed. The amounts of the acid in plants watered with a solution of sodium and potassium nitrate increased or decreased in proportion to the amount of nitrate used. RAVENNA and PELI think that nitrates and carbohydrates are necessary to the formation of the acid. TREUB agrees with these conclusions, and adds that dextrose is especially essential. The acid probably does not occur in plants as such, but in the form of a glucoside from which it can be liberated by an enzyme or by boiling water.—R. CATLIN ROSE.

Parasitic flagellates in plants.—Although rapid progress in the study of parasitic flagellates has shown them to be of widespread occurrence in animal organisms, the discovery of these parasites in plants is a noteworthy fact. The occurrence of a trypanosome-like parasite in the latex of *Euphorbia pilulifera* in Mauritius was first reported by LAFONT.[10] The discovery was soon afterward

---

[9] TREUB, M., Nouvelles recherches sur le rôle de l'acide cyanhydrique dans les plantes vertes. Ann. Jard. Bot. Buitenzorg II. **8**:84–118. 1910.

[10] LAFONT, A., Sur la présence d'un parasite de la classe des Flagellés dans le latex de *l'Euphorbia pilulifera*. Compt. Rend. Soc. Biol. **66**:1011–1013. 1909.

confirmed by DONOVAN[11] by observations on the same species of Euphorbia in Madras. LAFONT[12] now follows with a full account of the organism. The parasite, which was originally discovered in the latex of *Euphorbia pilulifera*, occurs also in the two other species *E. thymifolia* and *E. hypericifolia*. A search of the latex of some 50 other species of plants from various families failed to reveal similar organisms. About one-third of the *Euphorbia* plants from different stations were found to be infected. The number of parasites in different plants varies greatly. The infected plants show the effects of malnutrition, and finally drop their leaves and die. The protozoans are elongated, flattened, and somewhat undulate. They do not, however, possess the undulating membrane of trypanosomes, and are therefore placed in the genus *Leptomonas*, as *L. Davidi*. The apex is provided with one cilium, which originates in a blepharoplast. A large nucleus is situated near the center of the body. Division, which was observed in hanging drop cultures, takes place by longitudinal fission, preceded by a thickening of the body of the organism. Various forms, perhaps indicating different stages in the development of the organism, were observed. The simplest are spherical, nucleated masses of protoplasm, which soon form a cilium. It is possible that two parasites exist here. Injection of the parasites into the blood of small animals produced no infection, although some of the animals died from unknown causes.—H. HASSELBRING.

Diseases of celery.—KLEBAHN[13] has added to his numerous excellent contributions of life histories of *Fungi imperfecti* an account of two diseases of celery occurring in the truck gardens on the lowlands surrounding Hamburg. The first is the leaf-spot disease caused by *Septoria Apii* (Briosi and Cav.) Rostr., also known as *S. Petroselini* Desm. var. *Apii*, and as *Phlyctaena Magnusiana* (Allechr.) Bres. The fungus attacks the leaves, stems, and fruits of the celery plants, and forms pycnidia on all of these organs. In following out the manner in which the fungus lives through the winter, the author encountered no other fruiting stages. The fungus is carried over from year to year by means of spores which persist both in the pycnidia on the plant remnants left in the fields, and in the pycnidia on the seeds. With spores from both sources the author was able to produce infections on young plants with ease.

The second disease is a scab of the roots, which, although it has been reported from several places, has never been critically studied. The disease is shown to be due to a species of *Phoma*, for which the author here first uses the name *Ph. apiicola*, unfortunately without giving a technical description of the fungus.

---

[11] DONOVAN, C., Kala-azar in Madras, especially with regard to its connection with the dog and the bug (*Conarrhinus*). Lancet **177**:1495–1496. 1909.

[12] LAFONT, A., Sur le présence d'un *Leptomonas*, parasite de la classe des Flagellés dans le latex de trois Euphorbiacées. Ann. Inst. Pasteur **24**:205–219. *figs.* 7. 1910.

[13] KLEBAHN, H., Die Krankheiten des Selleries. Zeitschr. Pflanzenkrank. **20**: 1–40. *pls.* 2. *figs.* 14. 1910.

The pycnidia occur on the diseased roots, and more abundantly on the lower part of the petioles and on the fruit, but rarely on the leaves. Cultures were obtained from hyphae invading the sound tissue of the roots and from spores. The colonies from both sources were similar, and many infection experiments with mycelium from both sources on sound roots were successful. The action of this species of *Phoma* in producing a scab and rotting of celery tubers is a case analogous to the well-known root rot of sugar beets caused by another species of the same genus, *Ph. Betae*.—H. HASSELBRING.

Treatment for smuts.—The usual methods of treating seed-grain for the prevention of smuts have not proved applicable in the case of the loose smuts of wheat and barley, since these fungi persist through the winter, not by means of spores adhering to the surface of the grain, but by means of a dormant mycelium in the interior of the seed. APPEL,[14] following out the suggestion made by JENSEN at the time of the publication of his hot water treatment to use a preliminary treatment with cold water for seed infected with these smuts, has worked out a more definite method founded on an experimental basis. APPEL assumes that, like the spores of smuts, the dormant mycelium will start into growth more readily than the infected seed, and that the active mycelium will be killed at temperatures which do not injure the seed. The experiments substantiate this belief. It is found that grain infected with *Ustilago tritici* or *U. nuda* can be treated successfully by being soaked for six hours at 20–30° C., and by being treated subsequently with hot water at 50–54° C., or by hot air at a corresponding temperature.—H. HASSELBRING.

Dehiscence of anthers.—HANNIG [15] takes up what apparently he regards as a real difference between STEINBRINCK's cohesion theory and SCHNEIDER's *Schrumpfungstheorie* for the explanation of the dehiscence of anthers. To the reviewer the two theories differ more in the degree of analysis than anything else, as he believes that this phenomenon must be in the last analysis found dependent upon the tensile strength of water. However, the author has done a real service in showing how the dehiscence is a genuine cohesion consequence. He has accomplished this by artificially causing dehiscence through the effect of dehydrating solutions. He has shown that dehiscence will occur in a saturated atmosphere if anthers are exposed to light which generates enough heat in the tissues to reduce the vapor tension sufficiently to set up tension in the water contained in the membranes. BURCK's notion that the nectaries withdraw water from the membranes and hence cause dehiscence in a saturated atmosphere was not confirmed.—RAYMOND H. POND.

---

[14] APPEL, OTTO, Theorie und Praxis der Bekämpfung von *Ustilago tritici* und *Ustilago nuda*. Ber. Deutsch. Bot. Gesell. **27**:606–610. 1910.

[15] HANNIG, E., Ueber den Offnungsmechanismus der Antheren. Jahrb. Wiss. Bot. **47**:186–218. 1909.

Plant diseases.—WHETZEL and STEWART,[16] contrary to the common belief, advocate the cultivation of pear orchards if a crop of fruit is desired. In this view they are upheld by HEDRICK of the State Station, who found that blight epidemics are not necessarily dependent upon cultivation and manuring. No immunity to the disease was obtained by the use of certain blight remedies.

SACKETT[17] has described the appearance of this new bacterial disease in the field, and has given the manner of infection, together with a complete morphological, cultural, physical, and biochemical description of the causal organism, *Pseudomonas medicaginis* sp. n. The work is well supported by numerous inoculations. The only thing lacking in this well-balanced investigation is a bibliography.—VENUS W. POOL.

Source of nitrogen for molds.—RITTER[18] finds that the ammonium salts of mineral acids as the source of nitrogen for the molds is inverse to the strength of the acid forming the negative ion of the salt. The author attributes this to the toxic effect of the acid liberated by the assimilation of the ammonium ion. For instance, mono-ammonium or diammonium phosphate is a far better source of nitrogen than the ammonium salts of sulfuric, hydrochloric, or nitric acids. The so-called "Nitratpilze" (*Aspergillus glaucus, Mucor racemosus, Cladosporium herbarium*) gave on the average a greater yield of organic material from the two ammonium phosphates mentioned than from potassium nitrate. The yield from the ammonium salts of the stronger mineral acids was very much lower.—WILLIAM CROCKER.

Excretion of salts by Statice.—SCHTSCHERBACK[19] has investigated the excretion of salts by the leaves of *Statice Gmelini*. Many leaves of halophytes are known to excrete salts in considerable quantities by means of the glands described by DEBARY and others. Leaves of *Statice Gmelini*, floating on pure water, are soon freed from their contained salt and thereafter excrete water only. The amount of excretion of a leaf floating on a solution of a substance depends upon the substance and the concentration of it used; sulfates and chlorids of sodium, potassium, and magnesium tending to increase it, while calcium compounds and sugars decrease it. The amount of excretion does not depend upon the turgor pressure in the leaf cells.—R. CATLIN ROSE.

Physcia villosa in North America.—In a recent number of this journal (**49**: 320. 1910) I recorded this plant from southern California. Since then I have

---

[16] WHETZEL, H. H., and STEWART, V. B., Fire blight of pears, apples, quinces, etc. Bull. N.Y. Cornell Exp. Sta. **272**:31–51. *figs. 5–231.* 1910.

[17] SACKETT, W. G., A bacterial disease of alfalfa. Bull. Colo. Exp. Sta. **158**:1–32. *pls. 1–3.* 1910.

[18] RITTER, G., Ammoniak und Nitrate als Stickstoffquelle für Schimmelpilze. Ber. Deutsch. Bot. Gesell. **27**:582–588. 1909.

[19] SCHTSCHERBACK, JOHANN, Ueber die Salzausscheidung durch die Blätter von *Statice Gmelini*. Beih. Deutsch. Bot. Gesell. **28**:30–34. 1910.

found in the herbarium of Wellesley College a specimen distributed with *Evernia furfacea* (L.) Mann, collected by EDWARD PALMER at San Diego, California, in December 1888. A duplicate of this collecting has been kindly sent me by Dr. L. W. RIDDLE, who also calls my attention to the fact that this plant was distributed in *Decades North American Lichens* (no. 154) from San Quintin Bay, Lower California, Mexico, where it was collected by C. R. ORCUTT (see HASSE, Bryologist **13**:61. 1910).—R. HEBER HOWE, JR., *Thoreau Museum, Concord, Mass.*

Fertilization in Rafflesia.—The remarkable and renowned *Rafflesia* has long attracted attention, but little has been known of its more minute details. An investigation[20] of its embryo sac and fertilization shows that in spite of the parasitic habit and grotesque appearance, the development of the embryo sac and the process of fertilization are quite normal. It was noted that young stages in the development of the ovule are found in nearly mature buds, and that the development of the sac takes place after the flower is open.—CHARLES J. CHAMBERLAIN.

Microchemistry of chromosomes.[21]—The title arouses interest, but from the paper we learn only that chromosomes may be dissolved in hot water, while the reticulum of the resting nucleus is little affected, and that therefore the importance of chromatin in heredity has been overestimated. That there are chemical changes as chromosomes are developed from a reticulum has been known for some time, but we now know the effect of hot water upon chromosomes and theories of heredity.—CHARLES J. CHAMBERLAIN.

Absorption of salts by Bromeliaceae.—From his work with the Bromeliaceae, ASO[22] concludes that *Ananas sativus*, *Pitcairnia imbricata*, and *Nidularia purpurea* do not take up, or only in very small amounts, by means of the scales of the leaves, salts soluble in water. On the other hand, *Tillandsia usneoides*, after five days of submergence in a 0.3 per cent lithium nitrate solution, showed in different parts of the plant considerable quantities of the salt.—R. CATLIN ROSE.

---

[20] ERNST, A., und SCHMID, ED., Embryosack entwickelung bei *Rafflesia Patma* Bl. Ber. Deutsch. Bot. Gesell. **27**:176–186. *pl. 8.* 1909.

[21] NĚMEC, B., Zur Mikrochemie der Chromosomen. Ber. Deutsch. Bot. Gesell. **27**:43–47. 1909.

[22] ASO, K., Können Bromeliaceen durch die Schuppen der Blätter Salze aufnehmen? Flora **100**:447–449. 1910.